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Report to the Test Director

# GENETIC EFFECTS OF FAST NEUTRONS FROM NUCLEAR DETONATIONS


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January 1954


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OPERATION UPSHOT-KNOTHOLE, NEVADA PROVING GROUND, MARCH-JUNE 1953. FAST NEUTRONS FROM NUCLEAR DETONATIONS,

ABSTRACT

(U) A SERIES OF GENETRICS TEST OBJECTS WERE EXPOSED TO FAST-NEUTRON SURES WERE MADE BY PLACING THE SPECIMENS INSIDE 2-IN.-TH LEAD HEM SE. THESE OBJECTS WERE SET AT APPROPRIATE DISTANCES FROM OUND ZERO T MATERIALS WERE SUPPLIED BY A NUMBER OF ACTIVE INVESTIG RS AT OAK TO MERCURY IN THE PROPER STAGE FOR EXPOSURE SHORTLY BEFO THE SCHED ETURNED FOR BREEDING AND STUDY. PLANT MATERIALS USED INC D SPORES F DATURA AND OF CONR. DROSOPHILA FLIES, MORMONIELLA WASP AND SEVER SED. THE DATA SUPPORT THE FOLLOWING CONCLUSIONS: 1. THE LATIVE BIO M DETONATION NEUTRONS COMPARED WITH X OR GAMMA IRRADIATI IS HIGH, 2. THE RBE FOR CHROMOSOME BREAKAGE FROM DETONATION NEUT NS IS NOT ONS--THIS CONCLUSION IS SUBJECT TO SOME UNCERTAINTIES IN OSE DETERM ODUCTION OF SIMPLE MUTATIONS BY FAST NEUTRONS COMPARD WI X-RAYS TE MOSOME ABBERATIONS; 4. THE GENETIC TESTS ARE OF SOME VAL AS BIOLOG ; AND 5. THE STUDIES CLEARLY INDICATE THAT, FOR EQUAL DO S, NEUTRON OTHER FORMS OF RADIATION FROM NUCLEAR DETONATIONS,

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RELATIVE BIOLOGICAL EFFECTIVENESS
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SIMPLE MUTATIONS
TRADESCANTIA POLLEN
WASPS
7-IN THICK LEAD HEMISPHERES

## ABSTRACT

A series of genetics test objects were exposed to fast-neutron irradiation at Operation Upshot-Knothole. The exposures were made by placing the specimens inside 7-in.-thick lead hemispheres that were first used at Operation Greenhouse. These objects were set at appropriate distances from Ground Zero to cover the dose ranges required. The genetics test materials were supplied by a number of active investigators at Oak Ridge or at university laboratories and were sent to Mercury in the proper stage for exposure shortly before the scheduled detonations. After exposure the material was returned for breeding and study, and the summaries of the results were supplied for this report. Plant materials used included spores of several fungi, *Tradescantia* pollen, and seeds of *Datura* and of corn. *Drosophila* flies, *Mormoniella* wasps, and several mouse strains constituted the animal specimens used.

The data support the following conclusions:

1. The relative biological effectiveness (RBE) for genetic effects from detonation neutrons compared with x or gamma irradiation is high, observed values ranging from about 2 to 15 or more.

2. The RBE for chromosome breakage from detonation neutrons is not significantly higher than that from cyclotron neutrons; this conclusion is subject to some uncertainties in dose determination.

3. It is indicated that the RBE for the production of simple mutations by fast neutrons compared with x-rays tends to be low. The values are higher for gross chromosome aberrations.

4. The genetic tests are of some value as "biological dosimeters" supplementary to physical dosimetry.

5. The studies clearly indicate that for equal doses neutrons represent a greater potential genetic hazard than other forms of radiation from nuclear detonations.

## ACKNOWLEDGMENTS

It is difficult to acknowledge the assistance of everyone who aided in the genetics test program. Commander E. P. Cronkite and R. L. Corsbie gave continuous and patient help and counsel. Without the previous experience and the continuous help on the site of Dr. R. E. Carter, the test materials could not have been placed successfully. Dr. Alexander Hollaender was primarily responsible for the participation of the members of the Biology Division of Oak Ridge National Laboratory; their presence and sustained interest at the site were invaluable. Dr. J. W. Gowen of Iowa State College aided greatly in placing materials in all the tests. Dr. G. W. Beadle of the California Institute of Technology and Dr. Wilson Stone of the University of Texas were of material help in the program. Of course, the success of the genetics tests would have been impossible without the continued cooperation of the Test Organization and especially the personnel on the site.

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CHAPTER 1

# OBJECTIVES

The series of genetics tests at Operation Upshot-Knothole was planned to determine the frequencies of different kinds of mutations induced over a range of doses of fast neutrons in the detonations. The observed values were to be used to check the physical dosimeters and, more particularly, to determine the relative biological effectiveness (RBE) of fast neutrons compared with cyclotron neutrons and other forms of radiation in producing genetic changes of different kinds. The biological and genetic tests at Operation Greenhouse had already established that there were no qualitatively distinctive effects of detonation neutrons in spite of the obvious differences compared to laboratory sources. In addition, the Greenhouse tests showed that of the biological tests chosen in that series the most valuable as supplementary dosimeters were the thymus weight reduction in the mouse and chromosome breakage in the flowering plant *Tradescantia*. Since the aims for Operation Upshot-Knothole were broader, it was decided to set up a more inclusive series of tests emphasizing genetical studies and using most of the well-tested genetics materials. These were to be exposed to appropriate neutron dosages simultaneously under the conditions of the tests in order to be certain that the different biological effects were directly comparable.

It is important to establish the nature of the genetic effects of high-energy fast neutrons because the genetic changes induced by x-rays and gamma rays differ in important respects from other physiological effects produced by radiation. For instance, the length of life of mice is reduced by acute whole-body irradiation in relation to dosage, but the effect is relatively greater at higher dosages, and with decreasing doses the effect ultimately becomes so small that it can to all intents be considered negligible.[1] In contrast, mutations in the germ cells of flies are apparently produced according to a straight linear dosage vs mutation curve with no lower limit.[2] In addition, there is a dose-related recovery process for physiological effects of irradiation by chronic or repeated exposures to x-rays[3] but apparently no recovery for genetic changes. The nature of the dosage vs mutation curve has not been established with certainty for fast-neutron exposures.

There is good evidence for the conclusion that the biological effects of all radiations are produced by or closely related to ionizations within the cells traversed by the primary beam or the secondary particles. The differences between the physiological and the genetic effects may be determined by the nature of cells or tissues involved. The organism can recover from changes or lethal effects in a tissue if other unaffected cells remain to carry on the tissue activity. However, changed germ cells function as single cells in fertilization, and therefore the new individual formed by reproductive processes may be expected to show in all its cells whatever genetic effects the ionizations have produced.



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The higher efficiency of neutrons compared to x-rays in producing biological damage has been known for some time. It is clear that the effect is related to the higher density of ionization along the neutron recoil tracks as compared with the tracks of the recoil electrons produced by x-rays and gamma rays. Attempts have been made in the study of chromosome breakage and aberration production to explain the effect on the basis that the higher ion density has a better chance to produce primary chromosome breaks and that, in addition, as the ion density increases still higher the damage may reach a point where the healing of the broken ends is inhibited.[4] Because of these fundamental considerations, it was thought that confirmation of the high RBE for detonation neutrons would be an important objective. In addition, it was desirable to compare the RBE for the simple mutations which produce minimal chromosome disturbance with that for aberration production in which the effect of ion density on breakage and healing would play a greater role.

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CHAPTER 2

# EXPOSURES AND DOSAGES

Dosages for gamma rays were expressed in roentgens. For neutrons the roentgen equivalent physical (rep) was used. This is a dose of neutrons which is equal in energy absorption in tissue to 1 r of hard x-rays. The unit of effect is the roentgen equivalent man (rem), which is the number of roentgens of hard x-rays required to produce the same effect.

The tests of the genetic effects of fast neutrons at Operation Upshot-Knothole made use of the lead hemispheres first used at Operation Greenhouse and described in a Greenhouse report.[1] Since a large fraction of the fast neutrons passes through the 7 in. of lead to the interior chamber and an insignificant fraction of the gamma rays penetrates,[2] it had been assumed at both Greenhouse and Tumbler-Snapper that materials placed inside were being exposed almost entirely to a field of polyenergetic fast neutrons. But this assumption has had to be seriously questioned as a result of observations in the hemispheres at Operation Upshot-Knothole which suggest appreciable gamma-ray contamination. Nevertheless, the biological effects were due predominantly to the neutron component because of the high RBE of this radiation.

In defining RBE a distinction should be made between the relative effects from the two radiations for a given dose and the ratio of doses of x-rays to neutrons to produce a given effect. In this report the latter definition will be used throughout.

Neutron-flux measurements were made at the various hemisphere stations but do not yield dosage data. In all the experiments described in this report, sulfur and gold threshold detectors were used both inside and outside the hemispheres.[3] The sulfur detectors measured essentially all neutrons above about 3 Mev. The gold detectors were employed by the cadmium-difference technique to determine total thermal neutrons for detonation A. In detonations B and C, measurements of essentially total neutrons above thermal were made with boron-covered-plutonium fission detectors both inside and outside the hemispheres. The sulfur flux was thus shown to be a small fraction of the total neutrons. In detonation B, determinations of neutrons above about 1.5 to 2 Mev were obtained outside the hemispheres with uranium fission detectors provided by G. S. Hurst of the Oak Ridge National Laboratory (ORNL). These determinations alone were inadequate to establish neutron doses, since precise experimental information concerning the neutron spectrum inside the stations was lacking. Qualitatively, the neutron distribution in the hemispheres is probably quasi-exponential with a large fraction below 1 to 2 Mev. In detonation B the more rapid decrease of the uranium neutrons, compared with the total, suggested that the mean energy per neutron outside the hemispheres was declining at increasing distances from Ground Zero. Nevertheless, the sulfur neutrons did not decrease more rapidly than the total, suggesting that the small number of very fast neutrons did not disappear more rapidly than the other neutrons from the beam. These neutrons undoubtedly included a large component of particles which had made predominantly small-angle collisions with air molecules during their course from the shot point to the stations; only little energy is lost in such encounters.

The decline in average energy per neutron was also indicated in experiments with germanium neutron detectors provided by Dr. Benedict Cassen of the Atomic Energy Project at the University of California, Los Angeles (UCLA). These respond roughly in proportion to the total neutron energy incident on them. The germanium-detector readings declined more rapidly than the total neutron flux.

The estimates of total doses given in Table 2.1 were obtained from the readings of ionization chambers placed in the various stations. Chambers constructed of tissue-equivalent plastic were provided by Dr. H. H. Rossi, but their number was limited. Additional readings were provided by a larger number of small chambers with polyethylene walls.[4]

Table 2.1—DOSAGE SHEET FOR GENETIC EFFECTS OF NUCLEAR DETONATIONS A, B, AND C

| Stations | Neutrons, rep | Stations | Neutrons, rep | Stations | Neutrons, rep |
|---|---|---|---|---|---|
| Detonation A | | 23 | 1,400† | 20 | 94* |
| | | 24 | 70.5 | 22 | 71* |
| 3 | 35,000* | 25 | 45.5 | 23 | 46* |
| 4 | 18,000* | 26 | 21.5 | 24 | 38* |
| 5 | 7,000* | 27 | 15 | 25 | 27 |
| 6 | 4,000* | 28 | 9 | 26 | 19.2 |
| 7 | 3,500* | 29 | 1.3 | 27 | 14.3 |
| 8 | 2,500* | | | 28 | 6.1 |
| 9 | 2,000* | | | | |
| 10 | 1,700* | Detonation B | | | |
| 11 | 1,400* | | | Detonation C‡ | |
| 12 | 1,400* | 5 | 50,000* | | |
| 13 | 1,000* | 6 | 20,000* | 2 | 580 |
| 14 | 760* | 7 | 13,000* | 7 | 640 |
| 15 | 510* | 9 | 7,500* | 11 | 590 |
| 16 | 315* | 11 | 2,900* | 14 | 510 |
| 17 | 248 | 12 | 1,600* | 17 | 360 |
| 18 | 166 | 13 | 1,100* | 18 | 360 |
| 19 | 166 | 15 | 640* | 19 | 270 |
| 20 | 129 | 16 | 420* | 21 | 75 |
| 21 | 1,600† | 18 | 230* | 22 | 1,200† |
| 22 | 106 | 19 | 120* | 23 | 820† |

*Estimated by extrapolation.

†Essentially gamma rays.

‡Estimated by fitting a straight line on the log $RD^2$ plot to three Rossi-Failla dosimeter readings.

Readings of gamma-ray dose outside the stations were obtained with the National Bureau of Standards (NBS) film packs which had been used in Operation Greenhouse. Packs were also placed inside the stations to obtain gamma-ray estimates, but their partial sensitivity to neutrons limited their readings to an upper limit of the gamma-ray contamination of the neutron radiation in the stations. Some information concerning gamma rays was also obtained with Taplin chemical dosimeters, but again some neutron sensitivity has been shown for these instruments. Readings in detonation B were also obtained with a series of lead-lined ion chambers which should be preferentially gamma-ray sensitive; however, such chambers have some quality dependence, and the energy distribution of the gamma rays is not sufficiently well known to provide a satisfactory calibration for precise work. Readings varied from 13 to 38 per cent of the total dose.[4] Although the lead chambers were designed to be insensitive to neutrons, there may

be a small and uncertain neutron component to their readings. Two readings of carbon-lined chambers provided by Rossi indicated 13 and 23 per cent gamma-ray contaminations.

In analyzing the physical data, use was made of the fact that detonation neutrons, when corrected for the inverse-square effect, decrease quasi-exponentially with increasing distances from the shot point. Denoting the physical reading by R and the slant distance from the explosion center by D, plots of $RD^2$ on semilog coordinates were usually surprisingly straight over wide ranges of D with a few exceptions among the gold-flux curves. In detonation B a considerable variety of physical indexes were obtained. All were roughly parallel when plotted in this way. On the other hand, the linear regression line for the polyethylene ion chambers in this shot increased much more slowly toward D = 0. This suggested that the chambers were deficient in their ion collection at higher doses. The neutrons are delivered in a very small interval of time at a very high intensity, and under extreme conditions this can cause a glutting effect in the chambers which interferes with ion collection. One reading by a Rossi ion chamber at an inner station showed a considerably higher dose, suggesting that these chambers were less subject to the effect. In constructing the final dose estimates for this detonation, the values for the last three stations agreed with the Rossi values and were accepted as being correct. Extrapolation was then resorted to in which the $RD^2$ line was continued parallel to the line for the sulfur flux, and these values were used for the dosages reported in Table 2.1.

The situation was not as difficult in the case of detonation A. Here the dosimeter line on semilog coordinates was closely parallel to the sulfur-flux line, and so the readings were accepted up to the limit of the dosimeter capacities at station 25. Beyond this the doses were extrapolated parallel to the sulfur flux. In making these extrapolations, it was assumed without experimental proof that the dose follows the sulfur flux. Certainly, this is the only assumption which can be made at present, but it may easily lead to errors at close-in stations of as much as a factor of 3.

It is difficult to properly assess the amount of gamma-ray contamination in the hemispheres. The films are particularly unreliable at low doses as shown by the fact that at the farthest stations in detonations A and B the readings exceeded the total dose. Analysis of the log $RD^2$ plots also indicated definite deviations above the expected linear relations at low doses. All in all, the indications by the films of as much as 60 per cent gamma rays at some stations cannot be accepted since at least part of these readings can be attributed to slow-neutron effects. On the other hand, even in cyclotron experiments the gamma-ray component may easily be as much as 10 per cent. Taking Rossi's carbon chambers and the ORNL lead chambers at face value, the best estimate would indicate about 25 to 35 per cent gamma rays. Their presence does not influence the biological results very much; however, if the neutron component of the dose is to be obtained by difference from the total dose, the uncertainty in the gamma-ray component presents a serious difficulty. It should be noted that correcting for the gamma-ray effect will lower the neutron dose estimates, whereas the correction for incomplete ion collection will raise them.

It is evident from the foregoing considerations that the physical dose estimates in Operation Upshot-Knothole were not really very satisfactory. The rep values in Table 2.1 are given only because some numerical dose value is better than none. The problem of neutron dosimetry under the field conditions was a formidable one. Prior to this series of tests no physical dosimetry with neutrons had been seriously attempted. As a result of the work in Operation Upshot-Knothole, it is believed that the problem can be considered as partially solved for doses of a few hundred rep in future experiments, since the necessary refinements are now fairly well understood. The fact that at outer stations the physical doses and biological effects were in fair agreement with laboratory neutron experiments is in all probability much more than an accidental coincidence and suggests at least that approximately correct physical neutron dose estimates were achieved even if they were not established with conclusive proof.

Little information was available in advance of the tests concerning the correct placing of biological material to ensure the complete coverage of the optimum range of dosage. In order to work out the exposure schedule, use was made of the rem table for thymus weight losses worked out by Dr. R. E. Carter from the data obtained at Operation Greenhouse. This was

supplemented by preliminary physical studies in Oak Ridge by C. W. Sheppard and E. B. Darden of ORNL.[4,5] As a result of these preliminary studies and extrapolations from their curves and those of Carter, it was possible to estimate the expected doses and effects and to locate the biological material (from fungus spores to mice) in the hemispheres so that there would be a critical range of doses for the very first shot with little wastage.

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CHAPTER 3

# MATERIAL AND PROCEDURES FOR GENETICS TESTS

## 3.1 GENERAL PRINCIPLES

It is well known that the processes of inheritance in essentially all the unicellular and multicellular living things are determined by similar mechanisms. Small structures, the chromosomes, which can be seen at certain stages of cell division as threadlike bodies, bear along their length the genes whose composition governs the hereditary make-up of their bearer. Because of the uniformity of these processes of cell division and heredity, information concerning man can be obtained from the study of lower forms. In contrast to the science of human genetics, controlled experiments can be set up with lower forms which will give data having immediate human application. Different organisms have different advantages. Some, like *Drosophila* flies, reproduce rapidly in the laboratory, and cultures for two or more generations will give exact information about mutation rates. Others, like spiderwort plants, *Tradescantia*, have particularly large chromosomes which are useful for a study of breaks and aberrations. The choice is made on the basis of convenience for a particular study.

As a result of laboratory genetic experiments, it can now be said confidently that the exposure of man to ionizing radiation will certainly have a deleterious effect on subsequent generations. The object of present genetical experiments with radiation is to elucidate more clearly the nature and amount of this effect. The genetic data which are significant for the study of radiation effects on living organisms are rates of mutation. Mutations occur as changed genes or chromosome breaks in the chromosomes of germ cells, or in their precursor cells, and express themselves in observable differences in the embryo or adult characters of the offspring formed from these germ cells in subsequent generations. A mutation which causes the offspring to die before maturity is called a lethal mutation.

All the chromosomes are in pairs, one for each gene with the exception of one peculiar chromosome, the X chromosome, which is either single in the male or has a nonfunctional mate (the Y chromosome). Mutations in the X chromosome will be transmitted to daughters only and are called sex-linked mutations. In most organisms, including man, sexual reproduction determines that each individual possesses two of each gene, one received from each parent. If a mutated gene shows its effect in the offspring even when its mate is different, the mutation is said to be dominant. If, for the characteristic to appear, both genes must be of the same sort, the mutation is recessive. Lethal recessives will thus be carried in the population and will kill a descendant only when two appear by chance in the victim's cells. Thus, certain kinds of mutations will appear in the first generation, but recessive mutations may not express themselves until many generations later. The latter are still carried in the germ cells of certain individuals, however, and they accumulate as a reservoir of mostly deleterious genes which are only very gradually eliminated by selective processes.

For every species of plant or animal, including man, there is a constant natural or spontaneous rate of mutation which constitutes the basis for the natural variability and, with the forces of selection, the mechanism for evolutionary changes. Every species appears to be adjusted to this natural mutation rate in a dynamic equilibrium. The effect of radiation in sublethal doses is to increase the over-all mutation rate. This may be a significant hazard for the individual or for the species, dependent upon the sensitivity of the species to radiation and the spontaneous mutation rate. Some estimates of these levels have been summarized by Plough[1] and recently reviewed completely by Muller.[2]

The principal groups of organisms which have been most useful for genetic studies are fungi, some of the flowering plants, insects, and laboratory mammals. Since these show a wide range of radiation sensitivities, it was decided that all of them should be used for tests of the effects of fast neutrons in appropriate dosages at Operation Upshot-Knothole. Accordingly, the project leader asked a number of well-known geneticists who had been doing mutation studies with the various organisms if they wished to send properly prepared genetic material of their specialty for exposure to appropriate dosages of fast neutrons at Operation Upshot-Knothole. Certain individuals were invited to come to Mercury to aid in placing and recovering, but all were to make the experimental matings and tests at their own laboratories and report the results.

## 3.2 TESTS ON SPORES OF FUNGI (MOLDS, SMUTS, AND RUSTS)

Projects 23.5 (J. B. Rowell, University of Minnesota) and 23.12 (K. C. Atwood, ORNL) consisted of fungus material in the form of spores. Since most fungus spores are extremely resistant to radiation, Rowell's specimens were placed in detonation A in hemispheres closest to the zero point with exposures expected to develop 50,000 to 2000 neutron rep. Among the results to be expected was a table of survival percentages which could be used for determination of the RBE of survival after fast-neutron radiation in comparison with x-rays.

In Atwood's project, asexual spores (conidia) of the pink bread mold, *Neurospora crassa*, were exposed. Spores were used which possess more than one nucleus, and the stocks were chosen to be heterokaryotic, i.e., the cells contain two genetically different nuclei. *Neurospora* has the peculiar advantage for genetic study that mutants can be produced which appear biochemically as derangements of their metabolic machinery so that they are unable to synthesize particular necessary amino acids which normal wild-type molds can produce. If the growth medium then lacks these constituents, the cultures cannot survive. Mutants can thus be screened on the basis of growth behavior. Using heterokaryotic cultures, an ingenious demonstration can be made that radiation inactivates *Neurospora* by inactivation of the nuclei. In addition, Atwood exposed *Neurospora* spores in iron pipes at stations closer to the detonations than the lead hemispheres. This was to give a biological estimate of dosage outside.

## 3.3 PLANT CHROMOSOME BREAKAGE

Project 23.10 (J. S. Kirby-Smith and C. P. Swanson, ORNL) was concerned with exposure of mature pollen cells of the spiderwort *Tradescantia* to fast neutrons. *Tradescantia* pollen cells have few chromosomes, and these are large. They are thus well adapted to studies of chromosome breakage and rearrangement since the aberrations thus produced are readily seen under the microscope. The previous gamma-ray studies of Conger[3] at Operation Greenhouse established that this material constitutes an excellent biological dosimeter. The strain employed is well known for its uniformity of radiation response in different laboratories at various times of the year. In order to have the flower buds in the precise stage for critical exposures, it was desirable to transport the plants to Mercury several weeks in advance and grow them in cold frames in the area. *Tradescantia* pollen chromosomes are among the most sensitive indicators of radiation damage, and buds (inflorescences) were placed in the hemispheres farthest away from the explosion center at detonation A, with an expected range of 150 to 3 neutron rep.

Project 23.11 (A. F. Blakeslee, H. T. Yost, Jr., and J. L. Spencer, Smith College) tested both pollen chromosome breaks and pollen lethal mutations in the jimson weed, *Datura*. For this test *Datura* seeds, which are moderately resistant to radiation, were exposed. The seeds were planted after return to the laboratory, and the pollen formed in flowers on the plants which grew was studied. The planned seed exposures ranged from 8,000 to 250 neutron rep.

Project 23.16 (D. Schwartz, ORNL) was concerned with tests of corn (maize) seeds to determine survival values after germination and possible chromosome breakage. These seeds are resistant to radiation and were exposed over a wide range of dosages from possibly 20,000 to 100 neutron rep.

## 3.4 INSECT MUTATION TESTS

Most of the tests in insects were made using the classical genetic test species *Drosophila melanogaster* (the fruit fly), including Projects 23.4, 23.6, 23.7, and 23.8. This species is so well known genetically that detailed maps have been made of its chromosomes. It is known to be moderately radiation-resistant, and the tests were set up to cover a range of exposures of from 5,000 to 100 neutron rep.

Project 23.8 (J. W. Gowen, Iowa State College) was concerned with setting up a life table in relation to dosage indicating survival fertility, productivity, sex ratios, and sex-linked lethal mutations.

Project 23.4 (P. T. Ives, Amherst College) planned to tabulate for a dosage vs mutation curve several different kinds of mutations, including certain autosomal dominants, third chromosome recessives, and sex-linked lethals (autosomes are all the chromosomes other than the X).

Project 23.6 (G. H. Mickey and A. F. Yanders, Northwestern University, and W. K. Baker, ORNL) was concerned with dosage vs mutation frequency in *Drosophila*. The investigators studied mutation rates at certain specific loci in the chromosomes and also dominant lethals which would give results for the flies comparable with those found by Russell in the mouse.

Project 23.7a (E. M. Lewis, California Institute of Technology) made use of an original method for determining by genetic tests the number of chromosome rearrangements at a given chromosome location in *Drosophila*.

Project 23.7b (W. S. Stone, University of Texas), the last of the *Drosophila* projects, made use of methods developed at Texas for registering chromosome breaks in another fly species, *Drosophila virilis*. This gives an independent check on this important kind of mutation.

Project 23.9 (P. W. Whiting, University of Pennsylvania) used an entirely different insect, a parasitic wasp, *Mormoniella*, to test the mutation frequency in eye-color mutations. Wasps have a peculiarly convenient feature in that whereas the females have the usual pairs of chromosomes, i.e., they are diploid, the males are haploid, i.e., they have one only of each chromosome. As a result, mutations can be quickly checked in the immediate offspring of radiated females. X-ray data were available for comparison.

## 3.5 MOUSE EXPERIMENTS

The laboratory mouse has been widely used for testing effects of radiation-induced mutations and, being a mammal, provides a partial bridge in the wide gap between lower organisms and man. Another advantage with mice is that mating can be proved by inspection of the females for vaginal plugs. Typical of the similarities between mouse and man are the processes of genesis and maturation of sperm, and the manner in which lethality is reflected in preimplantation death or in death *in utero*. The embryology of the mouse is similar to that of man. The sensitivity of mice to radiation is also somewhat similar; thus a range of doses from 500 to 25 rep was planned.

Project 23.14 (J. W. Gowen, Iowa State College) arranged to expose animals from two strains with different radiation sensitivities in order to determine life tables of survival and productivity.

Project 23.13 (W. L. Russell, Liane B. Russell, and E. F. Oakberg, ORNL) made use of methods for determining mutation frequency, chromosomal aberrations, developmental effects, and testicular damage in mice. Prominence was given in the field test to the dominant lethal type of chromosomal aberrations because it was thought that, with the limited number of animals that could be exposed, this genetic effect would yield the most rapid and reliable quantitative data. By opening females that have been mated to irradiated males, the percentage of viable young, and young dead before or after implantation, can be quantitatively determined. Other chromosomal aberrations still being measured are those causing sterile or partially sterile offspring in the first generation. Gene mutation rates are also being determined by a method, described by W. L. Russell,[4] which has given the most significant results so far available on the genetic effects of x radiation of a mammal. All these studies are yielding information that is of immediate use in estimating the genetic danger to men from exposure to ionizing radiations.

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1. H. H. Plough, Radiation Tolerance and Genetic Effects, Nucleonics, 10(8): 16-20 (1952).
2. H. J. Muller, "Radiation Biology," Vol. I, Part I, Chap. 8, pp. 475-626, McGraw-Hill Book Company, Inc., New York, 1954.
3. A. D. Conger et al., Biological Dosimetry of Atomic Bombs, Using *Tradescantia*, Part III, Greenhouse Report, Annex 2.4, WT-43, September 1951.
4. W. L. Russell, X-ray-induced Mutations in Mice, Cold Spring Harbor Symposia Quant. Biol., 16: 327 (1951).

CHAPTER 4

# RESULTS OF EXPERIMENTS

## 4.1 FUNGI

Project 23.12 quantitative data were obtained by K. C. Atwood on survival in *Neurospora*. Two principal genetic characteristics were used as markers to identify the nuclei within the multinucleate cells. They were the requirement for arginine, i.e., a mutation in which the organism cannot synthesize this amino acid, and a second in which a requirement for methionine is coupled with a peculiar habit of growth of the colonies (amycelial). In the heterokaryotic spores, i.e., those having at least one each of the nuclear types, growth will occur on a medium lacking both arginine and methionine since the lack occurring in one nucleus is made up in the other. When, however, a spore contains two like nuclei (homokaryotic), it will not grow until its requirements are met by supplementation. Results for detonation A are shown in Table 4.1. From the survival percentages for the minimal medium (i.e., lacking arginine and methionine) the per cent of heterokaryotic cells was determined. By subtracting the number of heterokaryons from the number of cells growing on arginine media, the number of arginine-requiring cells was determined. Similarly, the number of methionine-amycelial cells was determined by subtraction from the cells growing on methionine media. Good agreement was obtained when the results were compared with those derived from observations of growth on media doubly supplemented by adding both arginine and methionine.

The fact that increased radiation causes a decrease in the heterokaryotic cells is of great interest. If radiation kills the cells by a general effect on the outer cell material (cytoplasm), then the percentage of heterokaryons will not change with radiation dose. On the other hand, if radiation inactivates cell nuclei, then single inactivations will be more frequent than multiple ones. If one member of a heterokaryotic pair is inactivated, the cell becomes homokaryotic since the two originally functioning, but different, nuclei no longer balance each other. The result will be a decrease in the fraction of heterokaryons and an increase in the homokaryotic fraction, which is seen in these data (Table 4.1). Although this principle has previously been shown for x radiation, it has not been demonstrated heretofore with predominantly neutron radiation. All the survival data obtained on *Neurospora* in Operation Upshot-Knothole showed these effects. The RBE for lethality cannot be given precisely, but it is about 4 to 5 for detonation A.

Colonies from the minimal plates in detonation A were isolated and tested for recessive lethal mutations in the amycelial component. The result is shown in Table 4.2. The difference between the total isolates and the number tested represents the number of isolates producing too few conidia (spores) to test. This difference is increased by radiation but is of no value as a measure of radiation damage in these experiments. The mutations other than lethals are a heterogeneous mixture of changes manifested in the homokaryotic amycelial component. These include slow growth, altered morphology, and unusual ratios of amycelial to normal (heterokaryotic) colonies. They are stable on transfer, but their genetic basis has not been investigated.

Table 4.1—SURVIVAL AND PROPORTION OF CELL TYPES IN *NEUROSPORA* EXPOSED IN DETONATION A

| Station | Minimal medium | Surviving fraction* | | | Fraction cell types | | | Est. dose, krep | Est. effect, krem† |
|---|---|---|---|---|---|---|---|---|---|
| | | Methionine | Arginine | Meth. + Arg. | Heterokaryotic | Homokaryotic Meth. | Homokaryotic Arg. | | |
| 5 | 0.15 | 0.47 | 0.43 | 0.55 | 0.15 | 0.49 | 0.36 | 7 | 38 |
| 6 | 0.39 | 0.68 | 0.68 | 0.80 | 0.29 | 0.39 | 0.32 | 4 | 19 |
| 7 | 0.51 | 0.77 | 0.74 | 0.88 | 0.35 | 0.37 | 0.28 | 3.5 | ~14 |
| 8 | 0.49 | 0.70 | 0.73 | 0.80 | 0.36 | 0.34 | 0.30 | 2.5 | ~14 |
| Control | 1.0 | 1.0 | 1.0 | 1.0 | 0.63 | 0.23 | 0.14 | | |

* Fraction of controls on same medium surviving.

† Estimated from equivalent dose of x-rays to produce the same survival. Doses less than 15 krem are too low for an accurate estimate.

Table 4.2—RECESSIVE LETHALS IN *NEUROSPORA* IN DETONATION A

| Station | Total isolates | Number tested | Per cent lethals | Per cent others | Per cent total |
|---|---|---|---|---|---|
| 5 | 310 | 296 | 9.5 | 4.4 | 13.9 |
| 6 | 360 | 356 | 2.0 | 1.4 | 3.4 |
| 7 | 360 | 358 | 1.7 | 2.2 | 3.9 |
| 8 | 360 | 358 | 1.7 | 1.7 | 3.4 |
| Control | 360 | 357 | 0.3 | 0.3 | 0.6 |

Table 4.3—SURVIVAL OF CONIDIA FROM VARIOUS MEDIA EXPOSED IN DETONATION B

| Station | Plating medium | Medium of origin | | | |
|---|---|---|---|---|---|
| | | $N^{15}$ | Normal | $N^{15}$, $B^{10}$ | $B^{10}$ |
| 5 | Minimal | 0.0083 | 0.0095 | 0.0019 | 0.0046 |
| | Supplemented | 0.14 | 0.11 | 0.059 | 0.083 |
| 6 | Minimal | 0.10 | Lost | 0.033 | Lost |
| | Supplemented | 0.73 | | 0.25 | |
| 7 | Minimal | 0.26 | 0.41 | 0.16 | 0.21 |
| | Supplemented | 1.34 | 0.93 | 0.75 | 0.85 |
| 9 | Minimal | 0.47 | 0.35 | 0.28 | 0.27 |
| | Supplemented | 0.79 | 0.48 | 0.38 | 0.57 |
| 12 | Minimal | 1.03 | 0.99 | 0.70 | 0.87 |
| | Supplemented | 0.89 | 1.10 | 0.71 | 0.80 |

Because there is a subjective element in scoring nonlethal mutants, the frequencies are not as reliable as those of the recessive lethals as a measure of radiation effect. The incidence of lethals in the present experiments and in the results from detonation B is somewhat lower than would be expected among conidia having the same surviving fraction after treatment with x or gamma rays.

After a preliminary experiment in detonation A, efforts were made in detonation B to influence the radiation effect by growing the cultures on media containing a varying distribution of elements with high slow-neutron-capture cross sections. In all of the media, a nitrate was used as the sole nitrogen source. In two of the media, a large portion of the nitrogen was $N^{15}$, the isotope with low capture cross section for thermal and epithermal neutrons. Two of the media were enriched with $B^{10}$ to the extent of 16 ppm. This is the isotope with high slow-neutron-capture cross section. A boron-free trace-element mixture was used in all. The results are given in Table 4.3. In these experiments control counts differed according to the medium of origin by as much as 20 per cent. This is interpreted largely as error due to differences in initial viability unrelated to the medium of origin. The heterokaryotic fraction remains essentially the same in all controls. In obtaining the surviving fractions, an average of controls for all four media was used. Any systematic error thus introduced will not introduce bias in the hypothesis that there is a slow-neutron effect.

Table 4.3 shows the survival of irradiated cells thus grown. The cases of survival greater than unity represent experimental error. Small differences in radiation effectiveness are much more easily detected at low survival than at high since the survival curves continually diverge. The survival of the boron-free material is consistently higher than that of the $B^{10}$ enriched. An effect of $N^{15}$ is not clearly apparent, however. No significant difference in the presence of $N^{15}$ or boron is seen in the recessive lethal results.

Data on survival and mutation were obtained from material placed at ground level in iron pipes outside the hemispheres in detonation B. The results are shown in Table 4.4. At two stations many of the samples contained no survivors, but since the total number of cells plated is known, an upper limit for the surviving fraction is given. There are several anomalous survival factors of unknown origin in some samples of this series. Of interest is the fact that there is little difference in the recessive lethal mutations inside and outside the hemispheres, whereas from the survival data the dose appears to be 4.5 to 6.0 times as great outside. X-ray doses to give equivalent effects in detonation B are given in Table 4.5. The RBE for lethality in detonation B is about 2 to 3 or approximately ½ that for detonation A. This illustrates the difficulty of dose estimation in the high-dose region.

Table 4.6 shows the data obtained from samples exposed in a nitrogen atmosphere. Several samples had to be discarded because of leakage, but two were satisfactory. Not only did nitrogen (or anoxia) fail to protect the material; it seemed actually to increase the radiation effect.

The preliminary conclusions from these studies are:

1. The RBE for the radiation in the hemispheres, compared with x-rays, is about 2 to 5 for the killing of *Neurospora* spores.

2. The biological dose at close-in stations was about 4.5 to 6 times greater outside the hemispheres than inside. The physical doses must differ by more than this owing to the lower effect of gamma rays relative to neutrons.

3. Boron ($B^{10}$) enrichment significantly decreased survival, indicating a small slow-neutron component of the effect.

4. A nitrogen atmosphere enhanced the effect of the radiation in the hemispheres, if anything.

Further conclusions must await the results of investigations now in progress.

Project 23.5 made use of a number of fungi of economic importance. For example, *Puccinia graminis* is the species of rusts on grain, *Phytophthora infestans* is the potato blight, etc. All the fungi that were used infect certain crop plants, and they were radiated in the stage in which they are spread from one plant to another, usually the spore stage. The survival could be tested in some cases by growing the irradiated spores on agar plates, but more often it could

Table 4.4—SURVIVAL OF CONIDIA EXPOSED OUTSIDE OF HEMISPHERES IN DETONATION B

| Station* | Plating medium | Medium of origin | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $N^{15}$ | Normal | $N^{15}$, $B^{10}$ | $B^{10}$ | $N^{15}$ | Normal | $N^{15}$, $B^{10}$ | $B^{10}$ |
| 5 | Minimal | $2\times10^{-7}$ | $2\times10^{-7}$ | $3\times10^{-7}$ | $3\times10^{-7}$ | | | | |
| | Supplemented | $1.1\times10^{-6}$ | $\sim8\times10^{-7}$ | $5\times10^{-7}$ | $6\times10^{-7}$ | | | | |
| 6 | Minimal | $8\times10^{-7}$ | $1.4\times10^{-6}$ | $9\times10^{-7}$ | $6\times10^{-7}$ | | | | |
| | Supplemented | $6\times10^{-6}$ | 0.0044 | $6\times10^{-7}$ | $10^{-7}$ | | | | |
| 9 | Minimal | 0.022 | 0.0090 | 0.0054 | 0.0018 | 0.057 | 0.030 | 0.0017 | 0.047 |
| | Supplemented | 0.16 | 0.12 | 0.044 | 0.046 | 0.43 | 0.26 | 0.020 | 0.11 |
| 12 | Minimal | 0.52 | 0.43 | 0.50 | 0.22 | 0.38 | 0.34 | 0.27 | 0.41 |
| | Supplemented | 1.07 | 1.14 | 0.89 | 0.44 | 0.70 | 1.20 | 0.94 | 1.01 |

* Locations were at same distance from Ground Zero as hemispheres.

Table 4.5—X-RAY DOSES NECESSARY TO GIVE EFFECTS EQUIVALENT TO STATIONS IN DETONATION B

| Station | Hemisphere | | Outside* dose range, krem |
|---|---|---|---|
| | Dose range, krem | Est. dose, krep | |
| 5 | 96–130 | 50 | >320 |
| 6 | 47–70 | 20 | 230–290 |
| 7 | 17–37 | 13 | |
| 9 | 15–23 | 7.5 | 63–110 |
| 12 | 0–5 | 1.6 | 16–33 |

* Stations at same location as hemispheres.

Table 4.6—CONIDIA EXPOSED IN $N_2$ ATMOSPHERE

| Medium of origin | Survival on | | Hetero-karyotic fraction | Total number isolates | Number tested | Per cent lethals | Per cent others | Per cent total |
|---|---|---|---|---|---|---|---|---|
| | Minimal medium | Supplemented medium | | | | | | |
| | | | Detonation A, Station 5 | | | | | |
| Standard | 0.90 | 0.54 | 0.12 | 120 | 112 | 18.8 | 7.1 | 25.9 |
| | | | Detonation B, Station 6 | | | | | |
| $B^{10}$ | $3.7\times10^{-4}$ | 0.014 | 0.017 | 25 | 22 | 31.8 | 13.6 | 45.5 |

be determined only by planting the irradiated material on the appropriate host plant and examining for subsequent infections.

The results obtained in detonation A are shown in Table 4.7 and for detonation B in Table 4.8. The most sensitive of the fungi was *Phytophthora infestans*, which showed retardation at about 1700 rep as given in Table 4.7 and was viable at about 1100 rep but dead at 2900 rep as shown in Table 4.8. Unfortunately, in the latter series the control was inviable. The death of all transfers of *Aphanomyces euteiches*, according to Rowell, should not be attributed to radiation.

The rusts, *Puccinia graminis tritici* and *P. graminis avenae*, were less sensitive to radiation. The germination of all samples was within normal limits, but the infectivity was depressed at about 7000 rep. Among the remaining fungi, resistance to radiation is high. *Melampsora lini* was depressed at 18,000 to 20,000 rep in both trials, but Rowell does not believe that the suggested depression of the *Ustilago* cultures in this region of dose can be considered as significant.

## 4.2 *TRADESCANTIA* CHROMOSOME BREAKAGE

The report for Project 23.10 (J. S. Kirby-Smith and C. P. Swanson, ORNL) as given by the investigators follows:

> *Tradescantia paludosa* inflorescences (buds) were exposed to radiation at detonation A, Operation Upshot-Knothole, in a number of the lead-hemisphere neutron stations. Material in stations 22, 24, 25, 26, 27, and 29 received doses in the ranges suitable for studies of chromosomal breakage. Slides were prepared from the exposed anthers at 24 hr and at 4 days after exposure, allowing both chromatid and chromosome aberration frequencies to be determined at a number of points.
>
> These data are summarized in Tables 4.9 and 4.10. The neutron doses in rep given here are final corrected figures derived from the Sheppard-Darden ion-chamber readings.[1] Rep values determined from dosimeters placed in the front and back positions in the hemispheres are indicated. Not enough physical data were obtained to derive a similar least-squares fitted set of doses for the back positions. The most reliable dose–aberration frequency curves are thus obtained from measurements made at the front positions. In the hemispheres containing appreciable numbers of mice, the *Tradescantia* chromosome aberration data indicate an attenuation in neutron dose from front to back of approximately 25 per cent. This can be clearly seen in the data for stations 22, 24, 25, and 26.
>
> In addition to the field test data, the tables contain the results of calibration studies made at Oak Ridge prior to the Nevada experiment. Considering *Tradescantia* as a biological dosimeter, the close agreement in the biological effects of cyclotron neutrons measured in rep at Oak Ridge and those due to detonation neutrons measured by ionization dosimeters in the lead hemispheres indicates that the uncertainties in the physical measurement of neutron dose in this dose range in the field are less than the factor of 2 conservatively set by Sheppard. This conclusion is based on the assumption that there is either little difference between the neutron energy spectrum within the lead hemispheres and the spectrum in the lead exposure chambers used in the cyclotron studies, or that the dependence of chromosome breakage on neutron energy over these ranges is slight. Although controlled laboratory experiments to determine the variation in chromosome aberration frequencies with incident neutron energy must be carried out before *Tradescantia* can function reliably as a neutron dosimeter, the present general agreement in dose determined in the field from biological and physical measurements is impressive.

These data were collected with the view of establishing the value of the *Tradescantia* material as an accurate biological dosimeter, and, together with Conger's data at Operation Greenhouse,[2] the usefulness of this plant for the purpose is now established. It was shown, in addition, that the values are closely similar to those found for similar rep exposures to cyclotron neutrons. Either differences in the energy distributions are unimportant or the spectra in cyclotron and field experiments were similar.

Although no data from x-ray exposures of *Tradescantia* were obtained at the site, the papers of Conger, and of Conger and Giles,[3] have already given such data, and their comparisons receive additional confirmation from the present tests. An estimate of the RBE from Conger's Greenhouse data for fast neutrons compared to x-rays yields the value 8 to 10. For simple one-hit chromatid and isochromatid breaks, an RBE of approximately 13 is indicated.

Table 4.7—SUMMARY OF THE EFFECTS OF NEUTRONS ON FUNGI, DATA FROM DETONATION A

| Fungus | Exposed cells | Hemisphere → / Dose, rep* → | Survival of total cells, % 0 | 14 760 | 10 1700 | 8 2500 | 5 7000 | 4 ~18,000 |
|---|---|---|---|---|---|---|---|---|
| **Rusts** | | | | | | | | |
| *Puccinia graminis tritici* | Race 11 urediospores | Germination | 40 | 43 | 51 | | 53 | 44 |
| | | Infection | Normal | Normal | Normal | | Sparse | Trace |
| | Race 56 urediospores | Germination | 56 | | 60 | 59 | | 48 |
| | | Infection | Normal | | Normal | Normal | | None |
| | Race 15B urediospores | Germination | 47 | 58 | | 37 | 42 | |
| | | Infection | Normal | Normal | | Normal | Sparse | |
| *Puccinia graminis avenae* | Race 7 urediospores | Germination | 15 | 19 | | 34 | | 10 |
| | | Infection | Normal | Normal | | Normal | | Trace |
| | Race 8 urediospores | Germination | 46 | | 39 | | 31 | |
| | | Infection | Normal | | Normal | | Sparse | |
| **Ascomycetes** | | | | | | | | |
| *Melampsora lini* | Urediospores | Germination | 5 | 3 | 0.3 | 0.3 | 0.0 | 0.4 |
| | | Infection | Normal | Normal | Normal | Normal | Normal | None |
| *Ustilago zeae* | 10A4-R1 sporidia | Germination | 61 | 59 | 64 | 38 | 38 | 28 |
| | | Colonies | 65 | 67 | 84 | 67 | 58 | 2 |
| | 17D4-C1 sporidia | Germination | 11 | 8 | 4 | 6 | 8 | 13 |
| *Ustilago avenae* | FeC4 sporidia | Germination | 6 | 60 | 67 | 10 | 42 | 37 |
| | | Colonies | 0.09 | 53 | 41 | 5 | 12 | 18 |
| *Sphacelotheca sorghi* | Chlamydospores | Germination | 92 | 89 | 88 | 78 | 86 | 86 |
| *Helminthosporium sativum* | Conidia | Dilutions | Normal growth throughout | | | | | |
| | Mycelium | Transfers | Normal growth throughout | | | | | |
| *Fusarium lini* | Conidia | Dilutions | Normal growth throughout | | | | | |
| | Mycelium | Transfers | Normal growth throughout | | | | | |
| *Diplodia zeae* | Conidia | Dilutions | Normal growth throughout | | | | | |
| | Mycelium | Transfers | Normal growth throughout | | | | | |
| *Rhizoctonia solani* | Mycelium | Transfers | Normal growth throughout | | | | | |
| **Phycomycetes** | | | | | | | | |
| *Phytophthora infestans* | Mycelium | Transfers | Normal | Normal | Retarded | None | Contam. | None |
| *Aphanomyces euteiches* | Mycelium | Transfers | (All cultures dead) | | | | | |

* Rep estimated by extrapolation.

Table 4.8—SUMMARY OF THE EFFECTS OF NEUTRONS ON FUNGI, DATA FROM DETONATION B

| Fungus | Exposed cells | | Survival of total cells, % | | | |
|---|---|---|---|---|---|---|
| | | Hemisphere → | | 7 | 6 | 5 |
| | | Dose, rep* → | 0 | 13,000 | 20,000 | 50,000 |
| Ascomycetes | | | | | | |
| *Melampsora lini* | Urediospores | Infection | Moderate | Moderate | Trace | None |
| *Ustilago zeae* | 10A4-R1 sporidia | Colonies | 2 | 9 | Contam. | 4 |
| | 17D4-C1 sporidia | Colonies | 35 | 14 | 8 | 15 |
| | 410qq-R1 sporidia | Colonies | 39 | 24 | 7 | 6 |
| *Ustilago avenae* | FeC4 sporidia | Colonies | 8 | 6 | 2 | 4 |
| *Helminthosporium sativum* | Conidia | Dilutions | 0.4 | | | |
| | Mycelium | Transfers | Normal growth throughout | | | |
| *Fusarium lini* | Conidia | Dilutions | 0.0053 | 0.014 | 0.0355 | 0.024 |
| | Mycelium | Transfers | Normal growth throughout | | | |
| *Diplodia zeae* | Conidia | Dilutions | Contam. | 0.0005 | 0 | 0 |
| | Mycelium | Transfers | Contam. | Normal growth | ---------- | |
| *Rhizoctonia solani* | Mycelium | Transfers | Normal growth throughout | | | |
| *Collectotrichum lini* | Conidia | Dilutions | 0.001 | 0.0008 | 0.0002 | 0.0005 |
| | Mycelium | Transfers | Normal growth throughout | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Hemisphere → | | 20 | 18 | 13 | 11 | 7 |
| | | Dose, rep* → | 0 | 94 | 230 | 1100 | 2900 | 13,000 |
| Phycomycetes | | | | | | | | |
| *Phytophthora infestans* | Mycelium | Transfers | Dead | Normal | Normal | Normal | Dead | Contam. |
| *Aphanomyces euteiches* | Mycelium | Transfers | ---------- | ---------- | ---------- | All dead | ---------- | ---------- |

*Rep estimated by extrapolation.

For those chromosomal aberrations which are not linearly related to x-ray dose, as they seem to be for neutrons, the RBE depends on dose. Taking these factors into account,[4] the RBE is about 7 for deletions and about 10 for exchanges.

Table 4.9— *TRADESCANTIA* CHROMOSOME ABERRATIONS

| Station | Dose, rep | Cells scored | Aberrations per 100 cells | |
|---|---|---|---|---|
| | | | Deletions | Exchanges |
| | | Detonation A | | |
| 22 (front) | 106 | 150 | 217 | 166 |
| 22 (back) | | 100 | 172 | 123 |
| 24 (front) | 70.5 | 200 | 140 | 130 |
| 24 (back) | | 200 | 128 | 102 |
| 25 (front) | 45.5 | 400 | 94 | 86 |
| 25 (back) | | 300 | 67 | 65 |
| 26 (front) | 21.5 | 200 | 33 | 37 |
| 26 (back) | | 300 | 26 | 31 |
| 27 (front) | 15 | 600 | 22 | 21 |
| 28 (front) | 9 | 600 | 15 | 15 |
| | | Oak Ridge Cyclotron | | |
| | 67.5 | 300 | 122 | 91 |
| | 56 | 300 | 94 | 79.5 |
| | 45 | 300 | 72 | 67.5 |
| | 22.5 | 300 | 39 | 32.5 |

Table 4.10— *TRADESCANTIA* CHROMATID ABERRATIONS

| Station | Dose, rep | Cells scored | Aberrations per 100 cells | | |
|---|---|---|---|---|---|
| | | | Chromatids | Isochromatids | Exchanges |
| | | Detonation A | | | |
| 27 (front) | 15 | 300 | 109.3 | 160.3 | 80.3 |
| 28 (front) | 9 | 450 | 51.5 | 82.7 | 37.1 |
| 29 (front) | 1.3 | 700 | 10.7 | 11.9 | 3.9 |
| | | Oak Ridge Cyclotron | | | |
| | 10.8 | 450 | 72.3 | 95.8 | 46.0 |
| | 5.4 | 250 | 37.6 | 40.2 | 17.2 |
| | 2.7 | 300 | 17.5 | 25.0 | 7.9 |

In general, then, detonation neutrons were about 10 times as effective as x-rays in producing aberrations in *Tradescantia*, the effects ranging from about 8 to about 13.

## 4.3 *DATURA* CHROMOSOME EFFECTS

The report of Project 23.11 (A. F. Blakeslee, H. T. Yost, Jr., J. L. Spencer, and Jean M. Cummings, Smith College Genetics Experiment Station) is summarized in Tables 4.11 to 4.14.

Table 4.11—EFFECT OF DETONATION NEUTRONS ON THE CHROMOSOMES OF *DATURA* *

| Station | Dose | No. of plants tested | No. of plants showing aberrations | Percentage aberrations | No. of chromosome breaks | Average no. of breaks per affected plant |
|---|---|---|---|---|---|---|
| | | | Detonation A | | | |
| | (rep) | | | | | |
| 5 | 7,000 | Lethal | | | | |
| 6 | 4,000 | Lethal | | | | |
| 9 | 2,000 | 55 | 32 | 59 | 88 | 2.7 |
| 11 | 1,400 | 47 | 16 | 34 | 36 | 2.2 |
| 14 | 760 | 56 | 12 | 21 | 29 | 2.4 |
| 17 | 248 | 24 | 3 | 12 | 5 | 1.7 |
| 22† | 106 + 1,500 r of γ rays | 8 | 2 | 25 | 4 | 2.0 |
| 23 | 1,400 r of γ rays | No data available | | | | |
| | | | Comparative Data | | | |
| | (r) | | | | | |
| X-rays | 10,000 | 23 | 4 | 17 | 10 | 2.5 |
| X-rays | 20,000 | 29 | 10 | 34 | 25 | 2.5 |
| γ rays | 10,000 | 42 | 8 | 19 | 22 | 2.75 |

* Approximate RBE = $\frac{\text{x-ray value}}{\text{neutron value}}$ = 15.

† Exposed inside a ¼-in. aluminum hemisphere.

Table 4.12—INDUCED POLLEN LETHALS IN *DATURA* *

| Dose | No. of plants tested† | Per cent with pollen lethals |
|---|---|---|
| Detonation | | |
| 7,000 rep | Lethal‡ | |
| 4,000 rep | Lethal‡ | |
| 2,000 rep | 107 | 93 |
| 1,400 rep | 217 | 86 |
| 760 rep | 334 | 59 |
| 248 rep | 262 | 21 |
| 106 rep + 1,500 r of γ rays§ | 255 | 9 |
| Gamma rays | | |
| 10,000 r | 68 | 64 |
| X-rays | | |
| 20,000 r | 21 | 76 |
| 10,000 r | 24 | 67 |
| Control | 218 | 0 |

* Approximate RBE = 12 below 10,000 r of x-rays.

† Two or more flowers per plant examined.

‡ Plants did not mature.

§ Exposed inside a ¼-in. aluminum hemisphere.

Table 4.13—TYPES OF INDUCED POLLEN LETHALS IN *DATURA*

| Dose | No. of flowers tested | Percentage pollen lethal types found in individual flowers: Chromosomal | Gene | Chromosomal and gene |
|---|---|---|---|---|
| Detonation A | | | | |
| 1,789 rep | 242 | 55 | 9 | 21 |
| 1,278 rep | 553 | 48 | 7 | 19 |
| 720 rep | 792 | 36 | 6 | 7 |
| 248 rep | 428 | 10 | 2 | 3 |
| 106 rep + 1,500 r of γ rays* | 503 | 4 | 2 | 2 |
| Gamma rays | | | | |
| 10,000 r | 163 | 24 | 15 | 7 |
| X-rays | | | | |
| 20,000 r | 60 | 50 | 2 | 15 |
| 10,000 r | 103 | 34 | 4 | 3 |
| Control | 594 | 0 | 0 | 0 |

* Exposed inside a ¼-in. aluminum hemisphere.

Table 4.14—INDUCED POLLEN LETHAL SECTORS IN *DATURA*

| Dose | No. of plants tested | No. of plants with sectors between: Chromosomal and normal types | Gene and normal types | Chromosomal and gene types |
|---|---|---|---|---|
| Detonation A | | | | |
| 1,789 rep | 107 | 9 (9%) | 1 (1%) | 31 (32%) |
| 1,278 rep | 217 | 16 (9%) | 6 (3%) | 83 (45%) |
| 720 rep | 334 | 44 (23%) | 6 (3%) | 25 (13%) |
| 248 rep | 262 | 16 (27%) | 5 (16%) | 2 (2%) |
| 106 rep + 1,500 r of γ rays* | 255 | 9 (43%) | 4 (28%) | 2 (11%) |
| Gamma rays | | | | |
| 10,000 r | 68 | 11 (25%) | 10 (23%) | 3 (7%) |
| X-rays | | | | |
| 20,000 r | 21 | 0 | 0 | 4 (30%) |
| 10,000 r | 24 | 9 (53%) | 2 (12%) | 1 (5%) |
| Control | 218 | 0 | 0 | 0 |

* Exposed inside a ¼-in. aluminum hemisphere.

These results are of interest because they show that another plant which appears to be capable of functioning as a dosimeter is available in addition to *Tradescantia*. The exposures are easier to make, but the results are available only after a longer period of time. *Datura* seeds contained in moisture-proof plastic bags were exposed in the various hemisphere stations at Operation Upshot-Knothole. They were returned immediately after exposure and planted. Chromosome examinations were made on the growing plants for the data in Table 4.11. When the plants flowered their pollen was examined and classified on the basis of previous studies of pollen form into normal, chromosomal lethal, and gene lethal. These classifications received a later check as described in Yost, Singleton, and Blakeslee.[5] Thus it

becomes possible to determine the relative frequencies of two kinds of mutations by the use of *Datura* in the same exposure, namely, chromosomal and gene mutations.

It is of special interest that the RBE for over-all chromosome aberrations from the data of Table 4.11 is about 15, as for the more sensitive of the *Tradescantia* effects. Thus the RBE shows a high value in a quite independent experiment using different plant material. Again the data show that chromosome breakage is more frequently produced by neutrons than by x-rays.

## 4.4 CORN SEED EXPOSURES

Project 23.16 (D. Schwartz, ORNL) was planned to develop data showing the effect of bomb neutrons on the behavior of chromosome breaks in maize. This study is not complete, and only preliminary data on seedling growth have been received. These preliminary findings are unexpected in that at the highest dosages the seedling growth was greater than that at dosages somewhat lower.

Table 4.15—GROWTH OF CORN FROM SEEDS IRRADIATED IN DETONATION B

| Station | Dose, krep | Average length of plumule, mm | Station | Dose, krep | Average length of plumule, mm |
|---|---|---|---|---|---|
| 5 | 50 | 26.1 | 13 | 1.1 | 240 |
| 6 | 20 | 16.4 | 15 | 0.64 | 240 (normal) |
| 7 | 13 | 11.6 | 16 | 0.42 | 240 |
| 9 | 7.5 | 16.8 | 18 | 0.23 | 240 |
| 11 | 2.9 | 165 | 19 | 0.12 | 240 |
| 12 | 1.6 | 190 | | | |

The results observed on seedlings from the irradiated corn are shown in Table 4.15. Measurements were made 9 days after planting in sand to ensure maximum germination. Germination was normal throughout. One hundred seeds were planted from each station. Seedlings in hemispheres 5 to 9 ceased growth about 6 days after planting. No mitotic figures were found in root tips of these seedlings, signifying growth by cell elongation only, rather than cell division.

At first these results were accepted with reserve, but irradiation with gamma rays in the laboratory confirmed the effect. A possible explanation is that the inversion of the relation between dose vs seedling length at high doses is caused by a depression by high doses of those biological factors (perhaps enzymes) which are responsible for the breakdown of plant tissue.

Further information is described in a separate report.[6]

## 4.5 STUDIES OF SURVIVAL, PRODUCTIVITY, GENE CHANGES, AND OTHER EFFECTS IN *DROSOPHILA* AND MICE

Project 23.8 (J. W. Gowen, Iowa State College) exposed males and females of several strains of *Drosophila melanogaster* in detonations A, B, and C. The results were complicated by statistical fluctuations caused in part at least by the difficulty in obtaining large enough numbers in any one series. However, qualitative or semiquantitative observations were obtained on several points, aided by combining data from all three shots and applying statistical analysis. The dose-effect relations were obtained by fitting linear regression lines to the data on semilog plots. This was suggested in part from early experience by Gowen and Gay[7] who found for very soft x-rays that the dose-effect relations were essentially linear. For such radiation the essentially single-hit type of curve should be produced at least over a limited dose range.

A similar situation is known for most neutron effects. Table 4.16 lists the effects studied and the percentage reduction per kilorep. The effect on fertility is about half that found for dominant lethals in irradiated males by Baker et al. (Project 23.6). The decline in sex ratio does not include a test of significance but agrees with the idea that dominant lethals can occur in the X chromosome. There is also evidence for the production of lethals and semilethals in the second generation, suggesting sex-linked recessives.

Table 4.16—RADIATION EFFECTS ON *DROSOPHILA*

| Effect | Per cent reduction per krep |
|---|---|
| Fertility | 40 |
| Survival of progeny | 43 |
| Reduction females/males | 7 |
| X chromosomes without lethals | 4 |
| X chromosomes without lethals or semilethals | 6 |
| X chromosomes without lethals, semilethals, or visibles | 7 |

Table 4.17 gives a list of the sex-linked changes observed by Gowen in *Drosophila melanogaster*. The results show that neutrons are more effective per unit dose (rep) than x-rays. His RBE for sex-linked lethals is about 2, and for progeny-produced and X-chromosome dominant lethals about 2 to 4.

In Project 23.14 effects on mice were studied. Principal emphasis was placed on mice placed in the Civil Defense shelters. A large part of the data involve simple lethality studies. Although neither lethality nor shelter studies properly belong in the program of genetics studies in the biomedical hemispheres, these additional results were of interest and will be presented briefly.

Table 4.18 gives the survivals of the mice for 1 week immediately after irradiation. According to Gowen:

> The 146 mice of strain S and the 139 mice of strain Ba survived the irradiation which took place in shelter 2. Seven mice in one particular place in the shelter died within 5 hr of the blast, the necropsies indicating they died from concussion or causes other than absorbed radiant energy. Shelter 2, as judged by this material, furnished adequate protection against the irradiation, although some change in design to protect against concussion may be desirable. This conclusion is borne out by the data on survival found in Tables 4.18 and 4.19. The mice from shelter 2 lived as well as the controls up to an age of 300 days. The 285 mice surviving the initial concussion lived even better than untreated mice in our other experiments. After 300 days from the blast 13 per cent of the shelter mice had died, whereas untreated mice of the same strains in other experiments have 13 to 20 per cent deaths in the same period. In terms of their relative life span, 300 days of life for a mouse would be comparable to 20 or 30 years after the irradiation exposure for a man. Table 4.20 shows that the reproductive rates of the shelter mice were fully equivalent to the controls. The conclusion to be drawn appears to be the hopeful one that shelters can be built to protect against irradiation damage from this source. It is my understanding that the physical measurements of radiant energy present in this shelter show that the irradiation dosages were low in amount, confirming the biological measurements.
>
> The measured dosages in rep as estimated in Tables 4.18 and 4.19 for the different stations in detonations A and C show that with increasing dosages there was a rapid decline in survival of the mice placed in the hemispheres. Between the doses from 0 to 75 rep all mice survived. For dose 166 rep, 36 per cent survived. For dose 270 rep, 38 per cent survived; for dose 360 rep, 3 per cent survived; and for the doses 510 rep and above, all the mice died. There was a sharp decline in the capacity of these 40-day-old mice to survive the atomic irradiations. The 50 per cent death point for these data would be roughly 75 to 150 rep, judged from graphical interpolation on the arithmetic log scale. For the data assembled by Plough, the point at which 50 per cent of the mice died when treated

Table 4.17—LETHALS, SEMILETHALS, AND VISIBLE GENE OR CHROMOSOME CHANGES IN THE SEX CHROMOSOMES OF *DROSOPHILA MELANOGASTER* FOLLOWING IRRADIATIONS OF DIFFERENT DOSAGES

| Detonation | Sex exposed | Station | No. of chromosomes tested | Lethals | Semilethals | Visibles |
|---|---|---|---|---|---|---|
| A | Male | 9 | 7 | 1 | 0 | 0 |
| | | 12 | 18 | 1 | 2 | 0 |
| | | 14 | 16 | 2 | 0 | 0 |
| | | 16 | 89 | 2 | 2 | 0 |
| | | 19 | 244 | 4 | 0 | 0 |
| | | 20 | 227 | 0 | 0 | 2 |
| | | 21 | 39 | 4 | 2 | 3 |
| | | 22 | 173 | 1 | 1 | 0 |
| | | 23 | 237 | 16 | 7 | 0 |
| | | Shelter | 64 | 0 | 0 | 0 |
| | Female | 12 | 3 | 0 | 0 | 0 |
| | | 19 | 64 | 0 | 1 | 0 |
| | | 20 | 5 | 0 | 0 | 0 |
| | | 21 | 57 | 2 | 0 | 0 |
| | | 22 | 92 | 0 | 0 | 0 |
| | | 23 | 10 | 0 | 0 | 0 |
| B | Male | 13 | 99 | 5 | 0 | 0 |
| | | 15 | 81 | 0 | 0 | 0 |
| | | 16 | 71 | 2 | 0 | 0 |
| | | 20 | 51 | 0 | 0 | 0 |
| | Female | 13 | 120 | 4 | 2 | 0 |
| | | 15 | 395 | 6 + 1? | 14 | 0 |
| | | 16 | 44 | 1 | 1 | 0 |
| | | 18 | 363 | 1 | 7 | 0 |
| | | 20 | 143 | 1 | 1 | 0 |
| | | Control | 151 | 1 + 1? | 0 | 0 |
| C | Male | 22 | 58 | 1 | 1? | 0 |
| | | 7 | 7 | 0 | 1 | 0 |
| | | 19 | 2 | 0 | 0 | 0 |
| | | 21 | 20 | 1 | 0 | 0 |
| | | Control | 1 | 0 | 0 | 0 |
| | Female | 2 | 131 | 1 | 2 | 0 |
| | | 22 | 298 | 1 | 4 + 3? | 0 |
| | | 7 | 101 | 0 | 4 + 1? | 0 |
| | | 14 | 2 | 0 | 0 | 0 |
| | | 21 | 44 | 0 | 0 | 0 |
| | | Control | 1 | 0 | 0 | 0 |

with x-rays was 500 r. If this is representative for our mice, the RBE is about 3 to 6. ... However, it is more than probable that the mice and *Drosophila* in the atomic experiments were exposed to much more unfavorable environmental conditions than those treated with x-rays.

Comparison of the strain resistances in Table 4.18 shows that the mice of strain S were more resistant than those of strain Ba. These differences are concordant with those observed for the strains when treated with x-rays. The effects of the two types of radiation parallel each other.

The life spans for mice exposed during detonations A and C, 15 or more days after exposure to the blasts, are given in Table 4.19. The observed deaths in groups exposed to relatively low doses of radiation are at about the same rate as in the controls and comparable with the deaths in the con-

Table 4.18—IMMEDIATE LETHAL EFFECTS OF NUCLEAR IRRADIATIONS MEASURED AS DEATHS OF 40-DAY-OLD MICE 1 WEEK AFTER IRRADIATION

| Location | Dose | Males | | | Females | | |
|---|---|---|---|---|---|---|---|
| | | Treated | Alive | Dead | Treated | Alive | Dead |
| | | Detonation A—S strain | | | | | |
| Control | | 7 | 7 | | 9 | 9 | |
| Shelter 2 (unmated) | Small | 65 | 65 | | 63 | 63 | |
| Shelter 2 (mated) | Small | | | | 18 | 18 | |
| Station 19 | 166 rep | 8 | 3 | 5 | 4 | 2 | 2 |
| Station 21 | 1600 r* | 6 | | 6 | 6 | | 6 |
| Station 23 | 1400 r* | 6 | | 6 | 6 | | 6 |
| | | Detonation A—Ba strain | | | | | |
| Control | | 8 | 8 | | 8 | 8 | |
| Shelter 2 (unmated) | Small | 64 | 64 | | 57 | 57 | |
| Shelter 2 (mated) | Small | | | | 18 | 18 | |
| Station 19 | 166 rep | 6 | 2 | 4 | 4 | 1 | 3 |
| Station 21 | 1600 r* | 4 | | 4 | 6 | | 6 |
| Station 23 | 1400 r* | 6 | | 6 | 6 | | 6 |
| | | Detonation C | | | | | |
| | | S strain—both sexes | | | Ba strain—both sexes | | |
| Station 22 | 1200 r* | 8 | | 8 | 16 | | 16 |
| Station 7 | 640 rep | 16 | | 16 (3)† | 16 | | 16 |
| Station 11 | 590 rep | 16 | | 16 | 16 | | 16 |
| Station 14 | 510 rep | 16 | | 16 (2)† | 16 | | 16 |
| Station 18 | 360 rep | 16 | 1 | 15 (7-1)‡ | 16 | | 16 |
| Station 19 | 270 rep | 16 | 12 | 4 (1-3)‡ | 16 | | 16 (3)† |
| Station 21 | 75 rep | 16 | 16 | | 16 | 16 | |
| Control | | 22 | 22 | | 16 | 16 | |

* Mostly gamma rays exposed inside aluminum hemispheres.

† Number in parentheses survived 1 week.

‡ To be read 7 survived 1 week and 1 survived 2 weeks; 1 survived 1 week and 3 survived 2 weeks.

trols. In other words, where the mice live for 15 days, they have equal chances for survival. The dose of irradiation to which the mice were exposed earlier seemingly has had only an acute effect, with recovery leading to a full life span.

The reproductive performances of the mice in the detonations are presented in Table 4.20. For the mice which had litters, the number of mice in these litters was not noticeably altered by the various exposures to the irradiations.

Sterility of the mice in the exposed groups involves genetical effects. It is shown in Table 4.20. All females exposed to radiation from the detonations have been sterile to date, whereas the control and shelter 2 females have been less than 5 per cent sterile.

The male sterility after the detonations was between 0 and 60 per cent in the range of 75 to 270 rep. These results, so far as the small numbers allow interpretation, indicate greater effects per rep than those observed in x-ray treatments per roentgen.

The progeny of all mice—controls, those exposed in shelter 2 to radiation from the detonations, and those exposed to measured dosages—have been necropsied and examined by Dr. W. F. Hollander for internal-organ as well as external changes which may deviate from normal mice. Mice examined included: 1331 progeny of controls, 9374 progeny from the mice exposed in shelter 2, 39 exposed to 166 rep in detonation A, 55 exposed to 270 rep in detonation C, and 226 exposed to 75 rep in detonation C. One type of deviation from normal has been observed. Animals of this type are gynandromorphs (sex mosaics). They occur in only one strain and are not due to any irradiation effects. As comparable material, more that 15,000 progeny of x-rayed parents have been examined and have failed to show any

Table 4.19—PARTIAL LIFE SPANS TO JAN. 1, 1954, OF MICE SURVIVING EXPOSURES TO DETONATIONS A AND C DISTRIBUTED BY LOCATION, STRAIN, AND SEX

| Type of treatment | Sex and strain | Days to death | | | | | | | | | | | | Survivors |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 15–40 | 40–65 | 65–90 | 90–115 | 115–140 | 140–165 | 165–190 | 190–215 | 215–240 | 240–265 | 265–290 | 290–315 | |
| **Detonation A** | | | | | | | | | | | | | | |
| Control | M, S | | | | | | | | | | | 1 | | 6 |
| | M, Ba | | | | | | | | | | | | | 8 |
| | F, S | | 1 | | | | | | | | | | | 8 |
| | F, Ba | | | | | | | 1 | | | | | | 7 |
| Shelter 2 | M, S | 2 | | 1* | | | | | 1 | 1 | | 1 | | 59 |
| Dose ~30 rep | M, Ba | | | 1 | 1 | | | 1 | 1 | | 2* | 1 | | 56 |
| | F, S | 2 | 2† | 2‡ | | | 1 | 1 | | | | 1† | | 57 + 15† |
| | F, Ba | 2†‡ | | | 2‡ | | 3§ | | 12¶ | 2 | | | | 49 + 3† |
| Station 19 | M, S | | | | | | | | | | | | | 3 |
| Dose ~166 rep | M, Ba | | | | | | | | | | | | | 2 |
| | F, S | | | | | | | | | | | | | 2 |
| | F, Ba | | | | | | | | | | | | 1 | 0 |
| **Detonation C** | | | | | | | | | | | | | | |
| Control | M, S | | | | | | | 1 | | | | | | 12 |
| | M, Ba | | | | | | | | | | | | | 8 |
| | F, S | | | | | | | | | | | | | 9 |
| | F, Ba | | | 1* | | | | | | | | | | 7 |
| Station 19 | M, S | | | | | | | | 1 | | | | | 5 |
| Dose ~270 rep | M, Ba | | | | | | | | | | | | | 0 |
| | F, S | | 1 | | | | | | | | | | | 5 |
| | F, Ba | | | | | | | | | | | | | 0 |
| Station 18 | M, S | | | | | | | | | | | | | 0 |
| Dose ~360 rep | M, Ba | | | | | | | | | | | | | 0 |
| | F, S | | | | | | | | | 1 | | | | 0 |
| | F, Ba | | | | | | | | | | | | | 0 |
| Station 21 | M, S | | | | | | | | | | | | | 8 |
| Dose ~75 rep | M, Ba | | | | | | | | | | | | | 8 |
| | F, S | | 1 | | | | | | | | 1 | | | 6 |
| | F, Ba | | 1 | | | | | | 1 | | 1 | | | 5 |

* Missing.
† Premated before treatment.
‡ 1 killed by accident.
§ 1 premated.
¶ All premated; 9 killed because of respiratory disease at 209 days.

deviations from normal of the types looked for in mice, even though the x-ray dosages have been severe enough to cause death. The progeny examined have shown no abnormalities. In this respect the mice exposed to the detonations do not differ from mice exposed to x-rays.

## 4.6 *DROSOPHILA* LETHAL AND OTHER MUTATIONS

Project 23.4 (P. T. Ives, Amherst College) was concerned with sex-linked lethal mutations and certain visible mutations at specific loci in the X chromosome of *Drosophila melanogaster*.

Table 4.20—REPRODUCTIVE RATES OF MICE SURVIVING NUCLEAR IRRADIATION; NUMBER OF LITTERS AND AVERAGE PROGENY PER LITTER TO JAN. 1, 1954, DISTRIBUTED BY LOCATION, STRAIN, AND SEX; MATINGS MADE 7 DAYS AFTER DETONATION

| Type of treatment | Sex and strain | Litters after detonation | | Average no. of litters | Per cent sterile |
|---|---|---|---|---|---|
| | | First | All | | |
| | | Detonation A | | | |
| Control | M, S | 7–6.6* | 39–7.1† | 5.5 | 0 |
| | M, Ba | 8–7.6 | 65–7.0 | 8.1 | 0 |
| | F, S | 8–6.2 | 40–7.2 | 4.4 | 0 |
| | F, Ba | 8–7.6 | 62–7.1 | 7.7 | 0 |
| Shelter 2 | M, S | 62–6.8 | 398–8.1 | 6.1 | 5 |
| Dose ~30 rep | M, Ba | 62–7.1 | 552–7.5 | 8.9 | 0 |
| | F, S | 60–6.8 | 396–7.8 | 6.2 | 5 |
| | F, Ba | 57–7.3 | 513–7.6 | 9.0 | 0 |
| | F,‡ S | 18–6.9 | 67–8.1 | 3.7 | 0 |
| | F,‡ Ba | 16–6.6 | 40–7.3 | 2.5 | 11 |
| Station 19 | M, S | 3–7.0 | 6–7.0 | 2.0 | 67 |
| Dose ~166 rep | M, Ba | 2–9.0 | 8–6.5 | 4.0 | 50 |
| | F, S | | | 0 | 100 |
| | F, Ba | | | 0 | 100 |
| | | Detonation C | | | |
| Control | M, S | 8–8.0 | 39–8.9 | 4.9 | 0 |
| | M, Ba | 8–8.9 | 49–10.4 | 6.1 | 0 |
| | F, S | 9–8.2 | 41–9.1 | 4.5 | 0 |
| | F, Ba | 6–7.2 | 34–6.5 | 5.7 | 13 |
| Station 19 | M, S | 6–6.3 | 27–9.5 | 4.5 | 0 |
| Dose ~270 rep | M, Ba | | | | |
| | F, S | 6–0 | 6–0 | | 100 |
| | F, Ba | | | | |
| Station 18 | M, S | | | | |
| Dose ~360 rep | M, Ba | | | | |
| | F, S | 1–0 | 1–0 | | 100 |
| | F, Ba | | | | |
| Station 21 | M, S | 7–8.8 | 37–9.9 | 5.3 | 13 |
| Dose ~75 rep | M, Ba | 8–7.7 | 46–10.7 | 5.7 | 0 |
| | F, S | 8–0 | | | 100 |
| | F, Ba | 8–0 | | | 100 |
| Premated | S | 12–3.0 | 3–3.0 | 3.0 | 75 |
| | Ba | 1–0 | | | 100 |

*Read: 7 litters, 6.6 mice per litter.
†Read: 39 litters, 7.1 mice per litter.
‡Premated.

Several of the methods used were similar to those used in Project 23.6, but it was considered desirable to have duplicate studies of this important problem.

In the study of recessive sex-linked lethal mutations, information is obtained from the fact that the X chromosome of the male is obtained from the mother and that the Y chromosome received from the father plays no role. The female receives an X from each parent. If the X chromosome contains a recessive lethal produced by irradiating the father, it will be covered in the female by the normal maternal chromosome and will not act. The male offspring receiving the X from the mother will live because it does not contain the lethal at all. In the

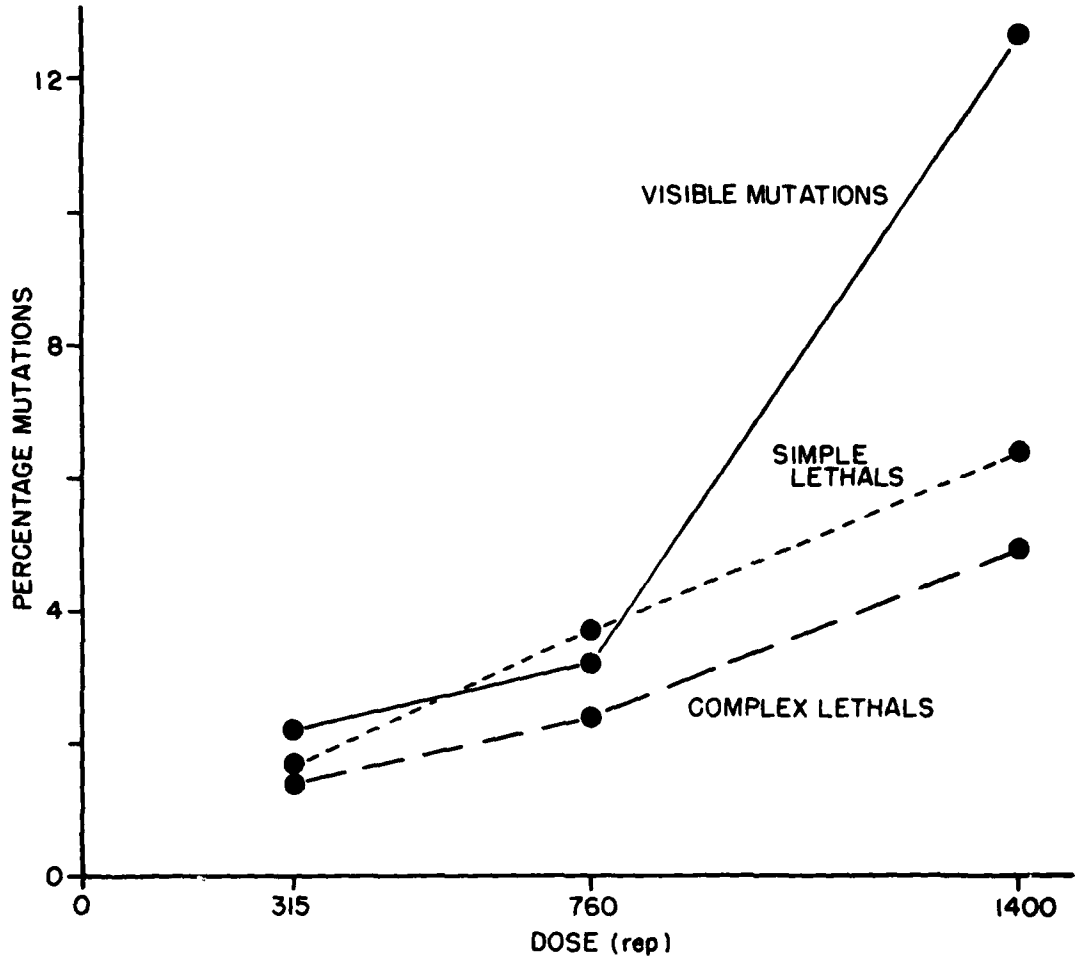


Fig. 4.1—Lethal mutations in *Drosophila melanogaster*.

second generation, however, the males all receive one or the other of the mother's two X chromosomes, and half the time they will receive the one with the lethal gene. There is no companion chromosome to cover up the effect, and so half the males will die. There would be nothing to add to this picture if it were not for the phenomenon of crossing over. This occurs during the special stages of cell division leading to formation of the reproductive cells (meiosis). Here a normal pair of chromosomes can exchange a portion of one another, provided they match correctly. However, if one contains a gross aberration, this match is interfered with and the result is a depression of the normal crossover frequency. This principle provides a method of preventing crossover if it is desired to carry flies from generation to generation in genetic testing. In identifying sex-linked recessives in *Drosophila*, special strains containing crossover suppressing aberrations, such as the Muller 5 or C1B, are used.

Although purposely suppressed in some genetic testing, crossover studies can be used to demonstrate aberration production in the irradiated stock. Such studies are conveniently done with special stocks containing marker genes at specific loci in the chromosomes. In Ives' work, four recessive genes were used at well-spaced points in the X chromosome. The genes involve external characteristics of the adult flies which are easily recognized. The first locus is one which controls body color. When both genes are recessive, the body will be yellow. If one or both genes are dominant, the body will have the normal wild-type grayish color. In addition to this yellow locus, called the *y* locus, the stock contains recessive pairs at the *ct* (cut wing), the *ras* (raspberry), and *f* (forked bristle) loci.

Table 4.21 — LETHAL MUTATIONS IN *DROSOPHILA*, X-CHROMOSOME DATA

| Part 1a | | | | Part 1b | | | |
|---|---|---|---|---|---|---|---|
| Percentage of Tests Showing Lethals | | | | Percentage of Lethals Having Chromosome Aberrations | | | |
| Dose | Tests | Lethals | Per cent | Tests | Aberrations | Per cent | |
| 315 rep | 1453 | 45 | 3.1 | 39 | 18 | 46 | Average, 42 |
| 760 rep | 1000 | 61 | 6.1 | 57 | 22 | 39 | |
| 1400 rep | 425 | 48 | 11.3 | 43 | 19 | 44 | |
| 2500 rep | 42 | 4 | 9.5 | 4 | 3 | 75 | |
| Soft x-rays (120 kv, 10 ma) | | | | | | | |
| 2500 r | 2403 | 165 | 6.9 | 40 | 6 | 15 | |

Part 2

| Proportion of Simple Lethals Vs That of Lethals with an Aberration, Among Total Lethals | | | | | |
|---|---|---|---|---|---|
| Dose | Per cent lethals | Simple lethals | Aberrations | Ratio: simple/aberration | |
| 315 rep | 3.1 | 1.7 | 1.4 | 1.2 | Average, 1.3 |
| 760 rep | 6.1 | 3.7 | 2.4 | 1.5 | |
| 1400 rep | 11.3 | 6.4 | 4.9 | 1.3 | |
| Soft x-rays (120 kv, 10 ma) | | | | | |
| 2500 r | 6.9 | 5.8 | 1.1 | 5.3 | |

Part 3

| Comparative Ratios of Simple Lethals to Lethals with a Chromosome Aberration | | |
|---|---|---|
| Source of mutations | Total lethals studied | Ratio: simple/aberration |
| Natural mutation gene | 351 | 19/1 |
| 2500 r, soft x-rays (120 kv, 10 ma) | 40 | 5.3/1 |
| High-intensity fast neutrons | 139 | 1.3/1 |

The lethal production obtained by Ives is shown in Fig. 4.1. Further data are given in Table 4.21. The complex lethals were determined as those of the total which inhibited crossing over 50 per cent or more, practically all cases being of this type. The simple lethals were the remaining fraction obtained by difference. Little information concerning the nature of the aberrations can be obtained until microscopic studies of the giant salivary-gland chromosomes of the flies can be made under the microscope. It is suspected that many may be translocations between the X chromosomes and other chromosomes.

With reference to these data Ives commented as follows:

> The results of Table 4.21, Parts 1b and 2, show that there was no change in the ratio, and in the proportion, of lethals associated with chromosome aberration as both the dosage and the frequency of lethal mutations increased. The average proportion was 42 per cent, not including the extremely small amount of data at 2500 rep, and the average ratio was 1.3. Presumably this means one hit caused the two breaks of the aberration in most cases.
>
> The data of Table 4.21, Parts 2 and 3, also make possible a rough comparison with 120-kv or soft x-ray effects[8] (though the data of the soft x-rays are small in size) and with the effects of a "natural mutation gene," which has the property of increasing the natural mutation rate in *Drosophila*.[9] The proportion of lethals with aberrations is definitely higher in the fast-neutron series as a group (315 to 1400 rep), the chi-square (with Yates correction) analysis yielding a P of only 0.003 in this case. The proportion of lethals with aberrations occurring from x-ray exposure is intermediate between the high proportion for neutrons and the low proportion for the natural mutation gene.

Table 4.22—DATA FROM THE "res" TESTS (VISIBLE MUTATIONS IN CHROMOSOME III)

| Dose | Flies | "res"-like* | Minutes | Other dominants | Unilateral mutations | All types, total |
|---|---|---|---|---|---|---|
| | | | Part 1: High-intensity Fast-neutron Series | | | |
| (rep) | | | | | | |
| 315 | 2,250 | 10–0.44 | 11–0.49 | 14–0.62 | 15–0.67 | 50–2.2 |
| 760 | 1,445 | 6–0.42 | 12–0.83 | 16–1.11 | 12–0.83 | 46–3.2 |
| 1,400 | 103 | 0 | 5–4.9 | 4–3.9 | 4–4.9 | 13–12.6 |
| 2,500 | 23 | 0 | 0 | 0 | 1–4.3 | 1–4.3 |
| Total | 3,821 | 16–0.42 | 28–0.73 | 34–0.89 | 32–0.84 | 110–2.88 |
| | | | Part 2: Soft X-rays Series (200 r/min) (120 kv, 10 ma) | | | |
| (r) | | | | | | |
| 3,000 or 3,200 | 13,301 | 68–0.51 | 135–1.01 | 62–0.47 | 146–1.10 | 411–3.1 |
| 5,000 | 5,130 | 32–0.62 | 87–1.70 | 44–0.86 | 78–1.52 | 241–4.7 |
| 7,500 | 672 | 12–1.79 | 17–2.53 | 13–1.94 | 21–3.13 | 63–9.4 |
| 10,000 | 393 | 3–0.76 | 16–4.07 | 11–2.80 | 18–4.58 | 48–12.2 |
| Total | 19,526 | 115–0.59 | 255–1.31 | 130–0.67 | 263–1.35 | 763–3.91 |

Part 3: $\chi^2$ Values

"res"-likes = 0.01
Minutes = 1.90
Other dominants = 9.78 (P for 1 df–0.0015)
Unilateral mutations = 0.83
*Sum of* $\chi^2$ *for 3 df* = 12.52 (P for 3 df–0.006)

(This is a comparison of 110 = 16 + 28 + 34 + 32 vs 763 = 115 + 255 + 130 + 263 for proportions of each type of mutation in the two series (Parts 1 and 2) and shows that there were proportionately more "other dominants" in the fast-neutron series.)

* Throughout, the first figure is the number of mutants, and the second is the percentage.

*Roughly 315 rep of fast neutrons gives* a proportion of lethals-with-an-aberration similar to that found in the 2500-r soft x-ray group, and 1400 rep of fast neutrons gives a proportion of simple lethals (without aberration) similar to that found in 2500-r soft x-rays. Considering both kinds of lethals together, 760 rep of fast neutrons gave a percentage (Table 4.21, Part 1a) similar to that found in the 2500-r soft x-ray series. Thus it appears that for the sex-linked data from chromosome I (the X chromosome) fast neutrons from the bomb produce simple lethals, or gene mutations with an RBE of about 2 as compared with soft x-rays, but gross chromosome aberrations show an RBE of about 8.

The study of visible mutations was made on chromosome III, which is not a sex chromosome, but one of the regular chromosomes (autosome). The test stock (res stock) contains a series of eight recessive marker genes. Normal wild-type males, if mated to res females, will yield offspring which are externally identical with their fathers because all the genes in the male are dominant. If a gene becomes mutated, it will almost invariably be recessively changed. Thus half the offspring will show a deviation from wild type in the body character which that gene controls. It is thus termed a "visible" mutation. Included among the visibles are the "minutes," which produce offspring reduced in size and are thought to be due to some sort of chromosome aberration.

Ives reported further as follows:

The data of Table 4.22 show a comparison of the visible mutation data of the fast-neutron series with several soft x-ray series. The solid line in Fig. 4.1 is the total from the last column of Table 4.22, Part 1, and differs from the other two curves in not fitting a straight line. There is a definite

suggestion in the small data for 2500 rep of fast neutrons that both lethals and visibles are less frequent than in the next lower dosage, 1400 rep. In the case of the solid line, if it had followed the others, it would have been no more than 6 per cent at 1400 rep. The difference between the expected and the actual one of 12.6 is statistically significant, P being about 0.01 from a Yates-corrected chi-square analysis.

The further comparison in Table 4.22, Part 3, suggests that fast neutrons produce proportionately more "other dominant" mutations than do soft x-rays, that being the only one of the four classes in which there was a proportionate difference between the two visible series. This may possibly be associated with the apparent greater frequency of chromosome breakage in the fast-neutron series suggested in the X-lethal studies of Table 4.21. Dominant visible mutations in radiation studies in general are very often associated with chromosome aberrations.

The unilateral mutations (also called "somatics") are an interesting problem in themselves, though apparently having a similar proportionate position in the visible mutations of both series, fast neutrons and soft x-rays. In these cases one-half of the fly was normal, the other mutant. In cases where the mutant was a "res"-like one, it was tested to the res stock. Several cases among those not showing the entire res-group of eight mutations gave about half mutant offspring and half completely normal offspring. That proved that in those cases the mutation was carried in some (but not all) germ cells, presumably from one gonad only. Other tests gave only res and + flies, with no single-mutant offspring. The unilaterals represent mutations produced by the radiation but delayed in occurrence for some time, until after the irradiated sperm had entered the egg and after several cell divisions had taken place. Very little attention has been given to the existence (and implication) of this kind of radiation-induced mutation in previous *Drosophila* work.

The data given in this report suggest that fast neutrons compared to x-rays give an RBE of about 2 for group 6 mutations and of about 8 for certain lethals which may be chromosome breaks. Here, even more clearly than by the data for Project 23.16, it is suggested that chromosome breaks are induced by neutrons at a higher frequency than gene mutations at the same dosages.

## 4.7 *DROSOPHILA* LETHALS AND MUTATIONS AT SPECIFIC LOCI

Project 23.6 (W. K. Baker, ORNL; G. H. Mickey and A. F. Yanders, Northwestern University) was planned to give data on dominant lethals and visible mutations at specific loci in chromosome III in *Drosophila melanogaster*. In addition, however, data on sex-linked recessives were obtained. The mutations at specific loci should give data comparable with Russell's data for the mouse. The latter portion of the project was partly duplicated in Project 23.4.

In the studies of mutations at specific loci, irradiated wild-type males (Oregon-R stock), from 2 to 4 days old, were mated to virgin res females.

Table 4.23 summarizes results obtained from the three types of experiments, x-ray, cyclotron, and detonation tests. Data from two x-ray experiments are combined, and the data from the detonation tests are pooled and calculated on the basis of rate per rep per locus $\times 10^{-6}$. These figures have been used to calculate the RBE of fast neutrons as compared to x-rays of 250 kvp, as shown in Table 4.24. Comparing the cyclotron effects with the x-ray mutations, a range is found from 1.2 to 17 at different loci, with an average RBE for all loci of 4. The value for *h* is quite high, whereas that for *sr* is low. The RBE's of neutrons from detonation tests as compared to x-rays range from less than 1 at the $e^s$ locus to more than 16 at the $p^p$ locus. The RBE for the average of all eight loci on chromosome III is 4.5. No statistical test of significance of the variations among the loci has been made, and the actual numerical values probably are not significant. However, the results are suggestive in a general way. Although the best available estimates of doses at the various stations were used, nevertheless the mutation rates and especially the RBE's at different loci must be considered as very crude approximations.

In the sex-linked lethal studies, irradiated wild-type males (Oregon-R stock), from 2 to 4 days old, were mated to virgin Muller-5 females.

Table 4.25 shows the results of experiments with the cyclotron, and Table 4.26 shows the results of detonation tests. In Fig. 4.2 these data are compared to those derived from x-ray experiments. It will be noted that the curves for lethals produced by the cyclotron and the

Table 4.23—MUTATION RATES AT SPECIFIC LOCI AND AVERAGE RATE FOR THE CHROMOSOME III MARKERS*

| Source of radiations | Dose | Sample size | Rates at specific loci per rep ($\times 10^{-6}$) | | | | | | | | Average rate per rep ($\times 10^{-6}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *ru* | *h* | *th* | *st* | *pp* | *cu* | *sr* | $e^s$ | |
| X-ray | 3000 r | 39,823 | (3) | (1) | (2) | (3) | (2) | (3) | (7) | (11) | (32) |
| | | | 2.51 | 0.84 | 1.67 | 2.51 | 1.67 | 2.51 | 5.86 | 9.21 | 3.35 |
| Cyclotron | 1000 rep | 15,260 | (2) | (2) | (1) | (0) | (3) | (3) | (1) | (3) | (15) |
| | | | 13.11 | 13.11 | 6.55 | | 19.66 | 19.66 | 6.55 | 19.66 | 12.29 |
| Detonations A and B | Various | 21,670 | (7) | (3) | (3) | (5) | (9) | (2) | (8) | (3) | (40) |
| | | | 20.93 | 8.97 | 8.97 | 14.95 | 26.91 | 5.98 | 23.92 | 8.97 | 14.95 |
| Control | | 18,802 | | | | | | | | | 0 |

*The figures in parentheses indicate the actual numbers of proved germinal mutants at each locus.

Table 4.24—ESTIMATED RBE OF FAST NEUTRONS AS COMPARED TO X-RAYS OF 250 KVP APPLIED TO SPECIFIC LOCI

| Source | RBE for specific loci | | | | | | | | Mean RBE |
|---|---|---|---|---|---|---|---|---|---|
| | *ru* | *h* | *th* | *st* | *pp* | *cu* | *sr* | $e^s$ | |
| Cyclotron | 5.65 | 16.89 | 4.14 | | 12.86 | 8.55 | 1.18 | 2.33 | 3.96* |
| Detonations A and B | 8.34 | 10.68 | 5.37 | 5.96 | 16.11 | 2.38 | 4.08 | 0.97 | 4.46 |

*Data corrected for 10 per cent gamma contamination.

Table 4.25—SUMMARY OF SEX-LINKED RECESSIVE LETHAL MUTATIONS INDUCED BY THE ORNL 86-IN. CYCLOTRON AT FOUR DIFFERENT DOSE LEVELS

| Dose, rep | No. of lethals | Chromosomes tested | Per cent lethal |
|---|---|---|---|
| 500 | 69 | 2118 | 3.26 |
| 1000 | 91 | 1393 | 6.53 |
| 1500 | 100 | 1091 | 9.17 |
| 2000 | 57 | 418 | 13.64 |

Table 4.26—SUMMARY OF SEX-LINKED RECESSIVE LETHAL MUTATIONS INDUCED BY NUCLEAR DETONATION TESTS IN DETONATION B

| Station | Estimated rep | No. of lethals | Chromosomes tested | Per cent lethal |
|---|---|---|---|---|
| 11 | 2900 | 13 | 96 | 13.54 |
| 13 | 1100 | 110* | 1918 | 5.74 |
| 15 | 640 | 88* | 2808 | 3.13 |
| 16 | 420 | 28 | 1314 | 2.13 |
| 18 | 230 | 7* | 546 | 1.28 |
| Control | | 3 | 1339 | 0.22 |

*Including some data contributed by W. K. Baker and E. S. Von Halle incorporated with our own by permission.

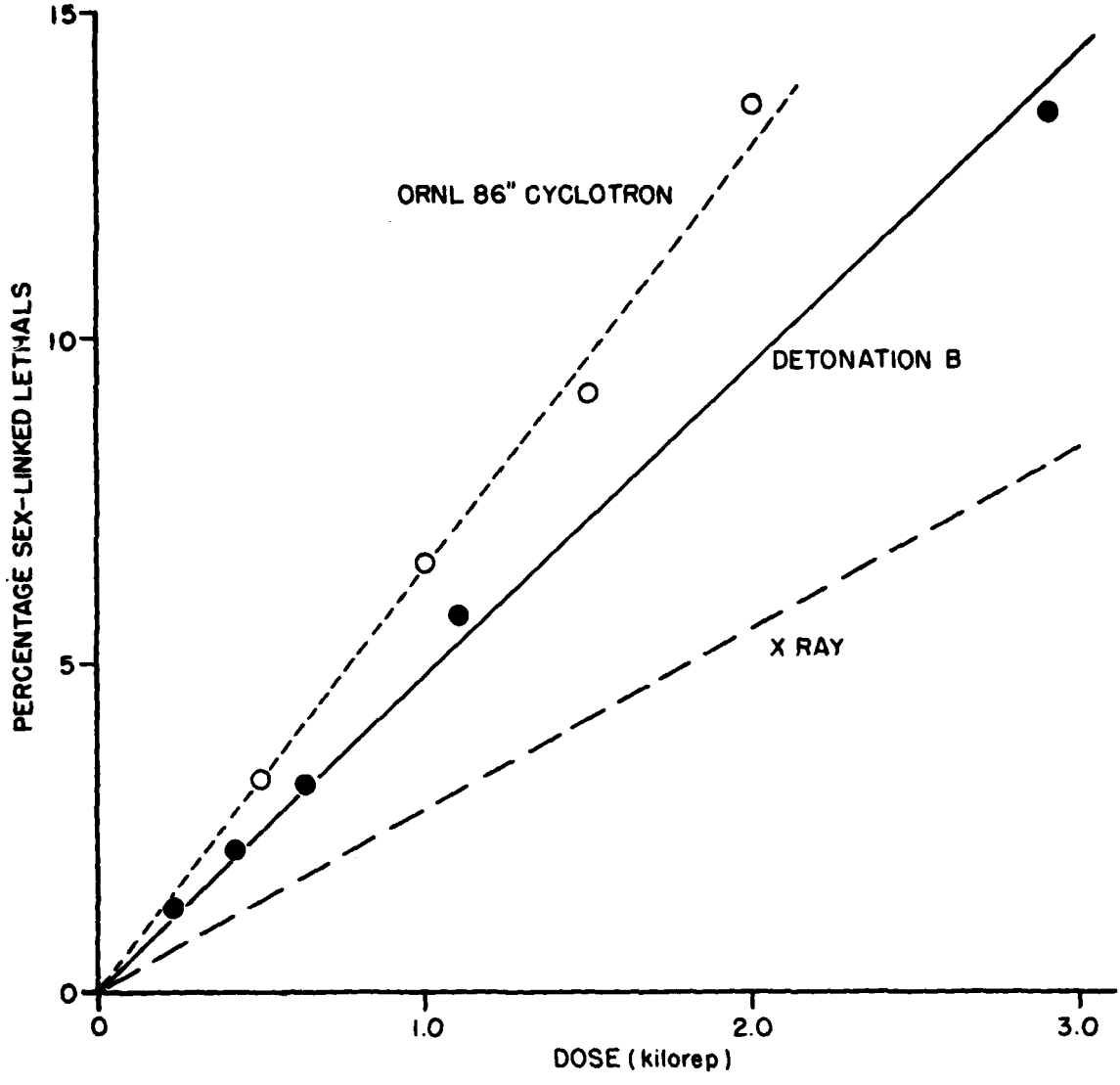


Fig. 4.2—Sex-linked lethal mutations in *Drosophila melanogaster.*

detonation are essentially linear. The slope for the detonation is about 0.7 that for the cyclotron. The difference in slope between both these curves and the x-ray curve indicates an RBE of neutrons as compared to x-rays of 1.7 for detonation B and 2.5 for the cyclotron. These values are less than the value observed for specific loci mutations and less than the lowest RBE observed by Baker for dominant lethals. Contrary to the general belief (based on studies of fast-neutron effects in producing sex-linked recessive lethal mutations) that fast neutrons are less effective than x-rays, the mutation rate in the groups exposed to fast neutrons was found to be significantly higher per unit of dose (rep).

The dominant lethal data from Baker showing a diminution in the percentage of flies which hatch in direct relation to dosage of irradiated parents indicate that lethal mutations (probably chromosome aberrations) are produced which kill the offspring in various stages of development. In the vast majority of these effects, death occurs before the hatching of the eggs.

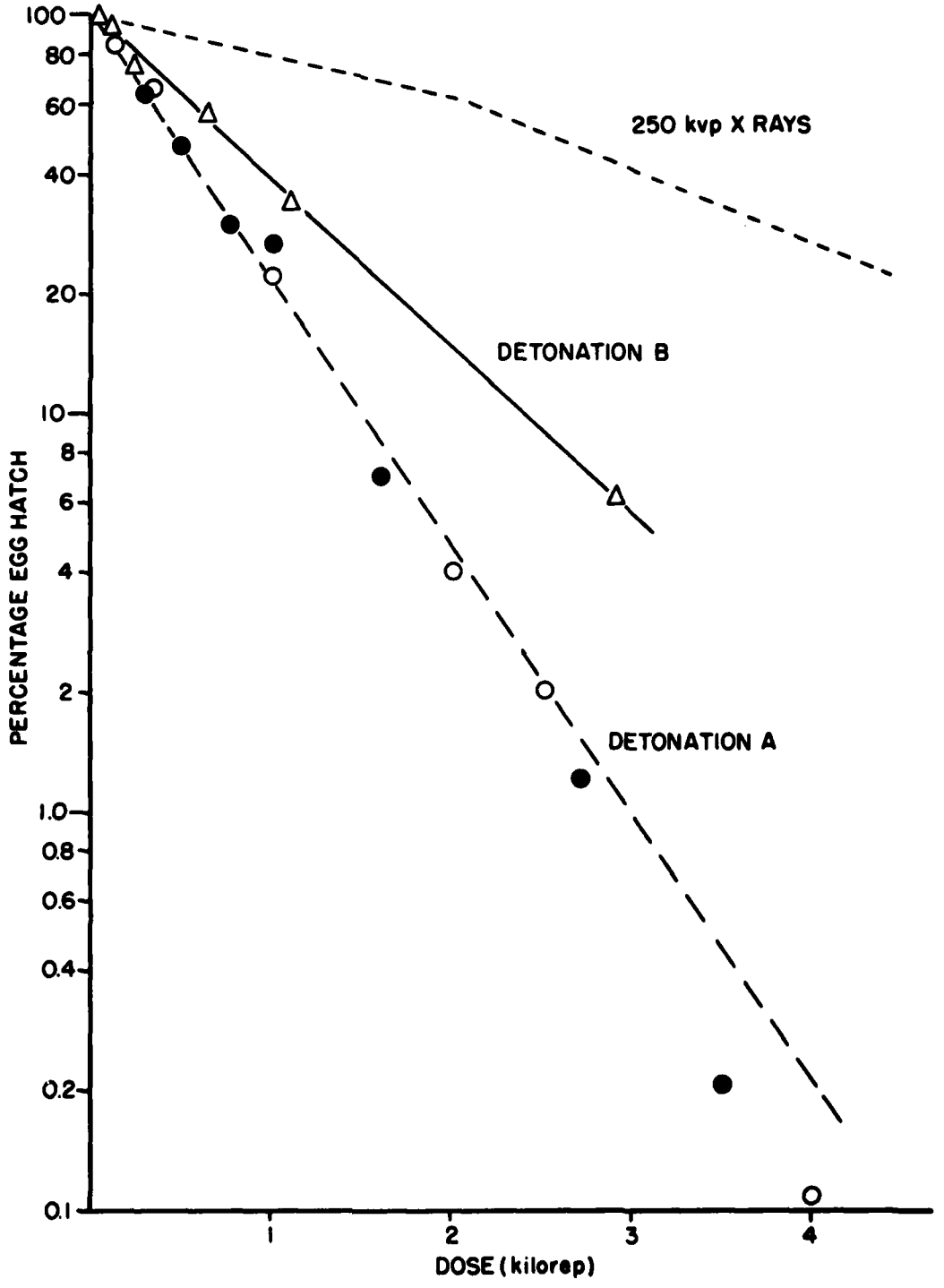


Fig. 4.3—Dominant lethal mutations in *Drosophila melanogaster*.

Oregon-R males were exposed and bred to untreated females of the same strain, and the percentage of eggs hatching was determined. The results are shown in Table 4.27 and Fig. 4.3. The cyclotron results are also shown in Fig. 4.3. The dosages for the two detonations are as given in Table 4.27. In a separate report Baker et al. used somewhat different dose estimates based on an alternative extrapolation procedure.[10] It is of interest that the cyclotron data and the results for detonation A are in close agreement. The fact that in detonation B the same effect (per cent hatch) occurs at a dose 1.6 times greater is as yet unexplained. Such large differences were not observed between the two detonations in the work of Stone in Project 23.7b. The RBE for detonation A ranges from about 4.7 to about 6.5 at the lower doses (0 to 1.5 krep). The variation is due to the fact that the neutron curves are essentially single-hit in nature, whereas the x-ray curves are multihit curves, and are not linear on the semilog plot. The RBE's for detonation B are about $^2/_3$ as great.

Table 4.27—DOMINANT LETHALITY IN *DROSOPHILA*

| Detonation A | | | | Detonation B | | | |
|---|---|---|---|---|---|---|---|
| Station | Dose, rep | Per cent hatch* | Total no. of eggs | Station | Dose, rep | Per cent hatch* | Total no. of eggs |
| 6 | 4000 | 0.11 | 1859 | 11 | 2900 | 6.32 | 2575 |
| 8 | 2500 | 2.04 | 1806 | 13 | 1100 | 34.4 | 2438 |
| 9 | 2000 | 4.06 | 4687 | 15 | 640 | 57.4 | 2663 |
| 13 | 1000 | 22.5 | 2660 | 18 | 230 | 75.6 | 1982 |
| 16 | 315 | 67.0 | 3325 | 20 | 94 | 94.1 | 2333 |
| 20 | 129 | 85.3 | 3428 | 24 | 38 | 97.8 | 2318 |

* Corrected for the control rate so that the control hatch would be 100 per cent.

## 4.8 CHROMOSOME REARRANGEMENTS IN *DROSOPHILA MELANOGASTER*

Project 23.7a (E. B. Lewis, California Institute of Technology) makes use of the ingenious bithorax method of testing for *Drosophila melanogaster* chromosome rearrangements by breeding tests.[11] These can be confirmed by chromosome examinations.

Lewis already had dosage vs mutation tests by this method made by exposure to x and gamma rays. Previous to the exposures at Operation Upshot-Knothole he also ran a series of exposures at Argonne. The source of fast neutrons was a fission plate covered with several inches of lead and exposed to reactor neutrons.[12]

These data (Tables 4.28 to 4.30) indicate that the high-energy neutrons in detonation A at Operation Upshot-Knothole produce very roughly the same number of rearrangements as do the pile neutrons (Argonne) at similar doses. In comparison with x- or gamma-ray effects, the RBE is approximately 5.3 to 6.7 between 3000 and 4500 r of x-rays. Although this is not as high as several values already indicated in this report, it gives support to the view that the biological effectiveness of fast neutrons is greater in producing chromosome breaks than in the production of gene mutations.

## 4.9 CHROMOSOME REARRANGEMENTS IN *DROSOPHILA VIRILIS*

Project 23.7b (W. S. Stone et al., University of Texas) was designed to test chromosome rearrangements in another species of fly, *Drosophila virilis*. The results of this project are summarized in Table 4.31 and Fig. 4.4. The report by W. S. Stone, M. L. Alexander, F. Clayton, and E. Dudgeon follows:

> The number of translocations produced in each hemisphere tested was directly proportional to dosage in rep as given in Table 2.1. Only one of the 11 tests for the two nuclear detonations was too

low to fit easily on the same straight line representing the relation between translocations produced and neutron dosage. We have indicated in Table 4.31 the maximum error from gamma-ray contamination assuming that the frequency of translocations from gamma rays would be equal to that of x-rays. We have included our genetic data for x-rays at 0°C in air delivered at 1818 r/min which were used in this correction on Fig. 4.4. Our data for x-rays and especially Baker's,[13] also using *Drosophila virilis*, show that the curves for translocations produced by x-rays usually result from two or more hits per rearrangement. These nuclear detonations give a one-hit curve for translocations (Fig. 4.4).

Table 4.28—PRODUCTION OF CHROMOSOME REARRANGEMENTS BY FISSION NEUTRONS AND FAST-NEUTRON EXPOSURES AT DETONATION A*

| Dose, rep | Total no. of flies | Percentage† of flies, grades 2 to 4 inclusive ±SE |
|---|---|---|
| | Argonne Fast-neutron Tests | |
| Control | 1303 | 0.0 |
| 200 | 1882 | 1.28 ± 0.26 |
| 400 | 2187 | 1.37 ± 0.25 |
| 800 | 1422 | 3.73 ± 0.50 |
| 1600 | 367 | 8.45 ± 1.4 |
| 3200 | 17 | 17.6 |
| | Detonation A | |
| Control | 398 | 0.0 |
| 106 | 877 | 0.34 ± 0.20 |
| 166 | 1179 | 0.76 ± 0.25 |
| 248 | 1151 | 0.96 ± 0.28 |
| 510 | 1246 | 1.69 ± 0.36 |
| 1000 | 709 | 4.80 ± 0.80 |
| 1700 | 215 | 5.58 ± 1.6 |
| 3500 | 12 | 16.7 |
| 4000 | 2 | |

* Comparison of results with the bithorax position effect method of detecting chromosomal rearrangements in *Drosophila melanogaster* using fast neutrons (treatment at Argonne, Jan. 22, 1953) at detonation A.

† Percentage of $F_1$ males showing a significant phenotypic departure from the controls in the bithorax effect; more than 90 per cent of the individuals falling into grades 2 to 4 have in progeny tests proved to carry chromosomal rearrangements, always with one break in a certain region of chromosome III (81-89).

The translocations are broken into classes: those involving two chromosomes ($T_2$), three ($T_3$), two independently segregating translocations ($T_{2+2}$ or $T_{2+3}$), and those involving four or five chromosomes in one complex translocation ($T_4$ or $T_5$). It is clear that the $T_{2+2}$ class is somewhat larger than the $T_4$ class. If broken chromosome parts rejoined at random (these were certainly all broken at once—at least all initial radiation damage is present at once), then we would expect half as many $T_{2+2}$ as $T_4$. Therefore the broken chromosomes do not rejoin at random but tend much more often to exchange two by two. This means calculations based on Haldane and Lea's paper[14] on expected combinations are incorrect in *Drosophila* as in *Tradescantia*.

The data show that for rearrangements of this type the RBE compared with x-rays is in the neighborhood of 3 to 6, depending on the dose range.

Table 4.29—DATA FROM X-RAY TESTS

| Dose, r* | Grade† 0 | Total | Per cent flies grades 2 to 4 | SD, % |
|---|---|---|---|---|
| Control | 2559 | 2563 | | |
| 200 (x-ray) | 694 | 698 | 0.3 | |
| 3000 (x-ray) | 2917 | 3020 | 2.32 | 0.27 |
| 3000 ($Co^{60}$ $\gamma$ rays) | 2274 | 2341 | 1.67 | 0.26 |
| 4000 (x-ray) | 3910 | 4085 | 3.38 | 0.28 |
| 4500 (x-ray) | 2679 | 2850 | 4.32 | 0.38 |

* All x-ray dosages given as 120 kv (8 ma) 15 cm from target (Westinghouse 140-kv industrial x-ray unit, with Machlett Radiographic type of tube).

† Phenotypic grade of $Bx^{34e}$ + / + Bxl flies.

Table 4.30—COMPARISON OF DOSAGE METHODS FOR DETONATION A

| Station | Gamma-ray contamination, r | Total dose, rep* | "Bithorax rep" units† |
|---|---|---|---|
| 22 | 60 | 106 | 70 |
| 18 | 90 | 166 | 150 |
| 17 | 120 | 248 | 190 |
| 15 | 340 | 510 | 330 |
| 13 | 610 | 1000 | 940 |
| 10 | 1200 | 1700 | 1100 |
| 7 | 3000 | 3500 | (3300)‡ |
| 6 | 4000 | 4000 | ‡ |

* See Table 2.1.

† Column 4 gives the calculated number of Argonne neutron rep that would have been required to give the same frequency of rearrangements that was observed at the designated test station. (Method of calculation: A least-squares linear plot of the Argonne fast-neutron data shown in Table 4.28 gives the equation $x = 19{,}507y$, using the 200-, 400-, 800-, and 1600-rep runs, where $x$ is the dosage in rep units and $y$ is the percentage flies in grades 2 to 4, inclusive. The observed values of $y$ from the test stations are substituted in that equation, and column 4 shows the value of $x$ obtained in each case.

‡ Insufficient data available owing to virtual complete sterility of the exposed flies.

Table 4.31—RADIATION DAMAGE TO GENETIC SYSTEMS: TRANSLOCATION

| Detonation | Station | Type of translocation $T_2$ | $T_3$ | $T_{3+2}$ | $T_4$ | $T_{2+3}$ | $T_5$ | All separate trans. | Normal | Total | Per cent separate trans. ±SD | Total dose, rep | Translocation $\times 10^6$/rep |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 7 | Genetic sterility—motile sperm in $P_1$ and eggs but not offspring | | | | | | | | | | 3900 | |
| A | 10 | 85 | 23 | 11 | 12 | 1 | 1 | 145 | 122 | 255 | 56.9±3.1 | 1700 | 335 |
| A | 13 | 134 | 28 | 14 | 7 | 2 | 1 | 202 | 352 | 538 | 37.5±2.1 | 1000 | 375 |
| A | 15 | 76 | 6 | 0 | 0 | 0 | 0 | 82 | 436 | 518 | 15.8±1.6 | 510 | 310 |
| A | 18 | 31 | 4 | 0 | 0 | 1 | 0 | 37 | 464 | 500 | 7.4±1.2 | 166 | 446 |
| A | 22 | 17 | 1 | 0 | 0 | 0 | 0 | 18 | 485 | 503 | 3.6±0.8 | 106 | 340 |
| B | 12 | 63 | 25 | 8 | 4 | 0 | 0 | 108 | 97 | 197 | 54.8±3.5 | 1600 | 343 |
| B | 13 | 148 | 26 | 7 | 7 | 2 | 0 | 199 | 359 | 549 | 36.2±2.0 | 1100 | 329 |
| B | 15 | 86 | 14 | 1 | 1 | 0 | 0 | 103 | 499 | 601 | 17.1±1.5 | 640 | 267 |
| B | 16 | 71 | 4 | 1 | 0 | 0 | 0 | 77 | 427 | 503 | 15.3±1.6 | 420 | 364 |
| B | 18 | 44 | 9 | 1 | 1 | 0 | 0 | 56 | 453 | 518 | 10.8±1.4 | 230 | 470 |
| B | 20 | 18 | 1 | 0 | 0 | 0 | 0 | 19 | 481 | 500 | 3.8±0.9 | 94 | 404 |
| B | 24 | Not run—too low | | | | | | | | | | | |
| X-rays | | 14 | 0 | 0 | 0 | 0 | 0 | 14 | 555 | 569 | 2.6±0.7 | 500 r | 52 |
| X-rays | | 49 | 0 | 0 | 0 | 0 | 0 | 49 | 809 | 858 | 5.8±0.8 | 1000 r | 58 |
| X-rays | | 109 | 10 | 2 | 0 | 0 | 0 | 123 | 582 | 703 | 17.5±1.4 | 2000 r | 87.5 |

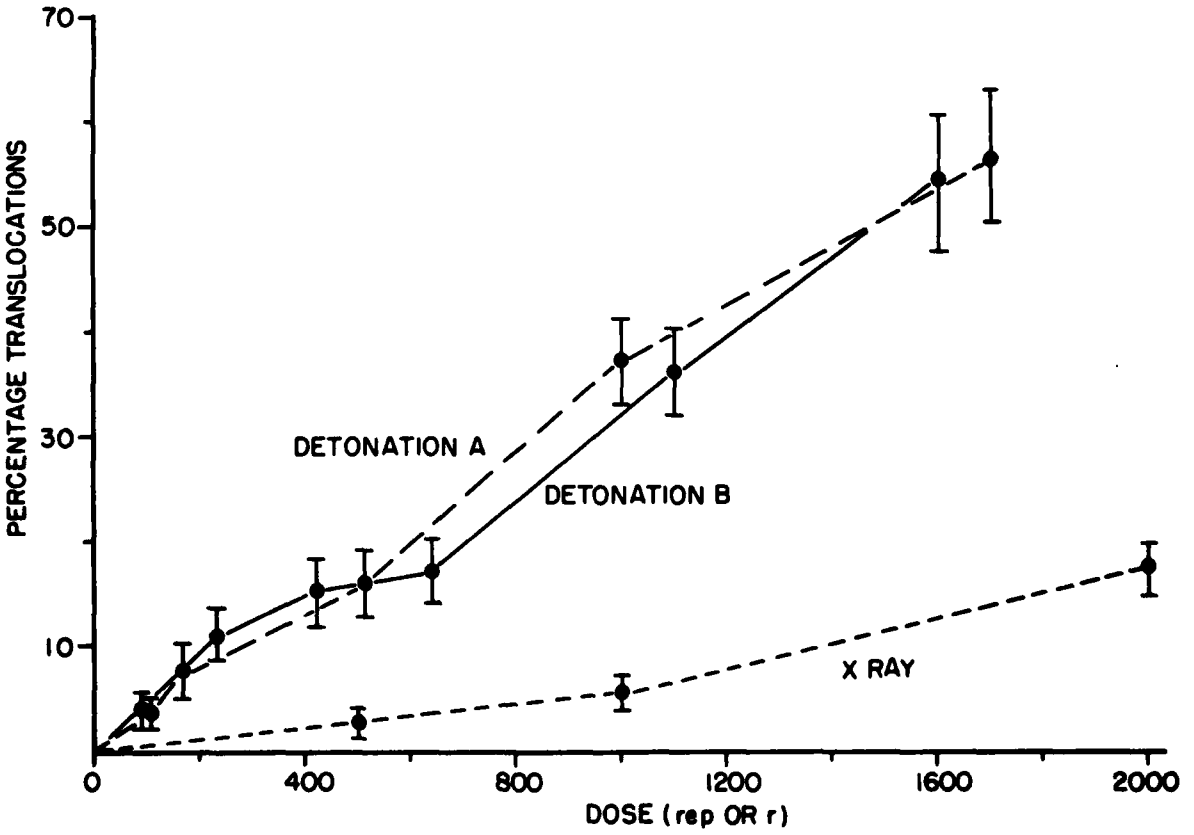


Fig. 4.4—Chromosome rearrangements in *Drosophila virilis*.

## 4.10 MUTATIONS IN THE WASP *MORMONIELLA*

Project 23.9 (P. W. Whiting and D. T. Ray, University of Pennsylvania) gives the number of eye-color mutations in the parasitic wasp *Mormoniella*, following exposures at three different detonations. The most extensive data were obtained in detonation A. Only two exposures were obtained in detonation C, and one of these was at an aluminum-covered station (No. 22) in which the dose was essentially all gamma rays. The results cannot be distinguished from gamma-ray effects. The results of the detonation tests are given in Table 4.32, and the x-ray mutation data are given in Table 4.33. The latter were obtained by Ray at the Marine Biological Laboratory, Woods Hole. The data in the two tables are shown graphically in Fig. 4.5. The

Table 4.32—MUTATIONS IN *MORMONIELLA* EXPOSED TO NUCLEAR DETONATIONS

| Hemisphere No. | Dose, rep | Bright-eye mutants, total sons | Per cent mutations | 95% confidence limits |
|---|---|---|---|---|
| | | Detonation A | | |
| 8 | 2500 | 4/159 | 2.5 | (0.6–6.4) |
| 9 | 2000 | 14/838 | 1.7 | (0.919–2.804) |
| 11 | 1400 | 29/2538 | 1.14 | (0.7644–1.639) |
| 14 | 760 | 18/7749 | 0.23 | (0.139–0.366) |
| 17 | 248 | 4/11,399 | 0.035 | (0.009–0.089) |
| 20 | 129 | 6/14,656 | 0.041 | (0.015–0.089) |
| | | Detonation C | | |
| 2 | 580 | 34/16,428 | 0.21 | (0.14–0.29) |
| 22 | 1200 r of $\gamma$ rays | 25/19,882 | 0.13 | (0.08–0.19) |
| | | Detonation B | | |
| 13 | 1100 | 91/12,591 | 0.723 | (0.589–0.883) |
| 15 | 640 | 64/22,647 | 0.28 | (0.22–0.36) |
| 16 | 420 | 44/16,449 | 0.27 | (0.19–0.36) |
| 18 | 230 | 12/9381 | 0.127 | (0.066–0.224) |
| 22 | 71 | 6/6737 | 0.089 | (0.033–0.194) |
| 23 | 46 | 4/6822 | 0.059 | (0.015–0.150) |

x-ray is essentially linear to about 2.5 krep, but in this region the frequencies in the detonation experiments rise in a quasi-exponential fashion. This apparent deviation from a single-hit type of curve is of interest since, in the range of dose normally studied in *Drosophila*, mutations increase linearly with the dose for x-rays and also seem to be linear for fast neutrons. Comparing the results for detonations A and B, the mutation frequencies seem, if anything, slightly greater in the second case. This is in contrast to Baker's dominant lethal results in *Drosophila*, where even in the low-dose range of 0 to 500 rep the effects in B were about $\frac{2}{3}$ those in A (Project 23.6). The RBE varies from about 3 in the low-dose range to about 5 at 1.3 krep.

## 4.11 DOMINANT LETHALS IN MICE

Project 23.13 (W. L. Russell, Liane B. Russell, and E. F. Oakberg, ORNL) included studies on genetic, developmental, and reproductive effects in mice. Some of the investigations are still proceeding. The preliminary report by W. L. Russell and Liane B. Russell on the dominant lethal effect follows:

The data from this experiment are now complete and are reported here in condensed form. A full account will be given in the final report. The main purpose of this investigation was to attempt to discover, for the effect under consideration, whether or not the high intensity and the particular spectrum of energies of neutrons from the detonation would show an RBE significantly different from that obtained in experiments with neutrons from laboratory sources.

The genetic effect measured in this experiment is dominant lethality in the offspring of exposed male mice mated shortly after irradiation. There has long been evidence that most of this type of lethality results from chromosome breakage in the spermatozoa or maturing gametes of the exposed males. The motility and fertilizing capacity of the sperm are not affected, but when a sperm damaged in this way fertilizes an egg, the cleavage divisions are abnormal and, sooner or later, the embryo dies.

Table 4.33—MUTATIONS IN *MORMONIELLA* EXPOSED TO X-RAYS

| X-rays, r | Bright-eye mutants, total sons | No. of mutations | Per cent mutations | 95% confidence limits |
|---|---|---|---|---|
| Low-dose X-ray Experiment by D. T. Ray, Summer 1953, MBL, Woods Hole | | | | |
| 0 | 149,908 | 4 | 0.0026 | (0.000667–0.00680) |
| 52 | 154,361 | 17 | 0.0110 | (0.00641–0.0176) |
| 105 | 154,580 | 21 | 0.0135 | (0.00840–0.0207) |
| 315 | 158,987 | 31 | 0.0195 | (0.0132–0.0276) |
| 525 | 148,992 | 52 | 0.0349 | (0.0262–0.0458) |
| Total | 766,828 | 125 | | |
| X-ray Experiment by D. T. Ray, Summer 1952, MBL, Woods Hole | | | | |
| 0 | 18,039 | 1 | 0.0055 | (0.00055–0.03104) |
| 1344 | 16,011 | 22 | 0.14 | (0.08–0.21) |
| 2688 | 10,058 | 25 | 0.25 | (0.16–0.37) |
| 4032 | 5,268 | 26 | 0.49 | (0.32–0.72) |
| 5376 | 2,708 | 23 | 0.85 | (0.54–1.27) |
| Total | 52,084 | 97 | | |

In order to reduce the gamma component of the radiation to a proportion that would not appreciably interfere with the estimation of neutron effects, the animals were exposed in lead hemispheres of 7-in. wall thickness and 14-in. inside diameter. The experimental material consisted of 144 young adult 101xC3H hybrid male mice. Twelve were exposed in each of 10 hemispheres placed at various distances from the detonation. The remaining 24 animals, used as controls, were placed in hemispheres 2 days before the detonation and for a length of time approximately the same as that required for the exposed animals. All animals were returned to Oak Ridge, and 1½ days after the detonation each male was placed with four adult untreated females of the same strain. The uteri of females that were pregnant from matings made from 2 to 6 days after irradiation of the male were removed at a late stage in pregnancy, and the uterine contents were examined. At the same time the number of corpora lutea in the ovaries of each of these females was recorded. The results are given in Table 4.34.

Table 4.35 shows the results obtained from a comparable cyclotron experiment in which males of the same strain of mice were exposed in a lead chamber of 2-in. wall thickness to fast neutrons from a beryllium target placed in the proton beam of the ORNL 86-in. cyclotron. The Victoreen dosimeter used was calibrated against three "tissue-equivalent" ion chambers. Gamma-ray contamination was estimated by a bismuth ion chamber to be about 10 per cent of the total dose in rep. Details of dosimetry are given by Sheppard and Darden.[16]

It is pertinent to the main purpose of the investigation to compare the ages, at death, of the affected embryos in the cyclotron and detonation results. First, it is apparent from Tables 4.34 and 4.35 that, in both sets of data, death after day 10½ of gestation is negligible. Second, although the relative percentages of death occurring before and after implantation vary with dose, they are not significantly different in the cyclotron and detonation results for comparable levels of total effect. It

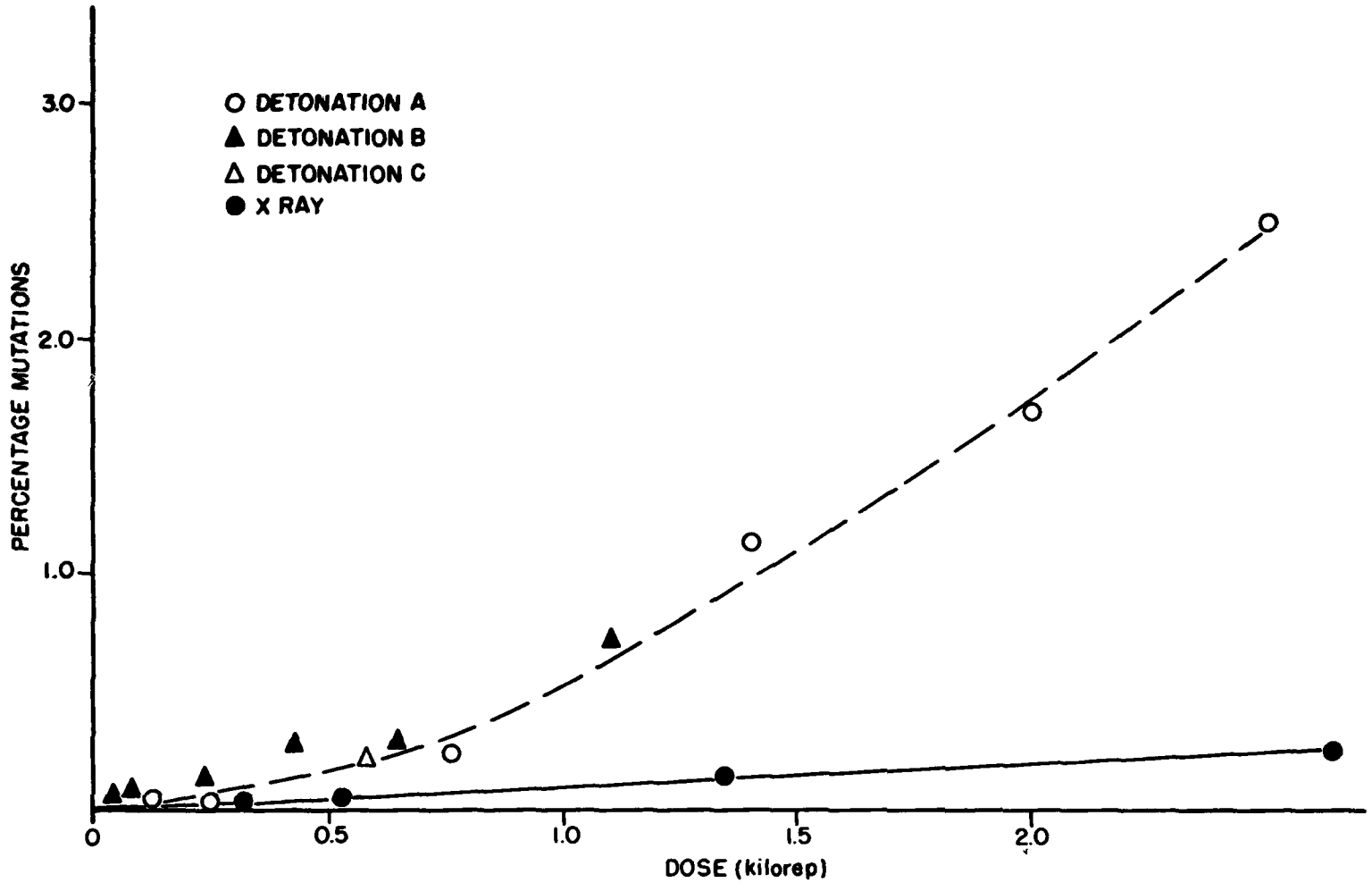


Fig. 4.5—Eye-color mutations in *Mormoniella*.

Table 4.34—DOMINANT LETHALITY IN OFFSPRING OF MALE MICE MATED FROM 2 TO 6 DAYS AFTER EXPOSURE TO NUCLEAR DETONATION A

| Lead-hemisphere exposure station | Minimum estimate of total dose (ion-chamber readings), rep | Maximum estimate of dose of γ rays (film dosimeter readings corrected for gold and sulfur neutron effects),* r | No. of pregnancies | No. of corpora lutea | Percentage of corpora lutea represented by | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Living fetuses | Embryos or fetuses dying after day $10\frac{1}{2}$ | Embryos dying between implantation and day $10\frac{1}{2}$ | Eggs or embryos dying before implantation (100 minus sum of cols. 6, 7, and 8) | "Survivors" (sum of columns 6 and 7) |
| (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) | (10) |
| Control | 0 | 0 | 48 | 446 | 84.30 | 0.45 | 3.59 | 11.66 | 84.75 |
| 26 | 22 | 11 | 21 | 201 | 73.13 | 2.49 | 7.46 | 16.92 | 75.62 |
| 25 | 50 | 25 | 22 | 208 | 60.58 | 0.48 | 19.71 | 19.23 | 61.06 |
| 24 | 83† | 33 | 22 | 214 | 50.47 | 0.47 | 21.03 | 28.04 | 50.93 |
| 22 | 96 | 47 | 25 | 249 | 42.57 | 0.00 | 26.10 | 31.33 | 42.57 |
| 20 | 131 | 62 | 21 | 198 | 34.85 | 0.00 | 27.78 | 37.37 | 34.85 |
| 18 | 157† | 67 | 24 | 232‡ | 32.82 | 1.30 | 25.91 | 39.97 | 34.12 |
| 17 | 266.5† | 83 | 17 | 155‡ | 23.22 | 0.00 | 24.51 | 52.28 | 23.22 |
| 16 | | 120 | 8 | 80‡ | 8.74 | 2.50 | 21.23 | 67.53 | 11.24 |
| 15 | | 266 | 6 | 57‡ | 0.00 | 0.00 | 13.99 | 86.01 | 0.00 |
| 14 | | 426 | 2 | 19‡ | 0.00 | 0.00 | 10.49 | 89.51 | 0.00 |

*The true gamma dose is probably much lower. The film response to neutrons of intermediate energies has not yet been reported.

†Mean of two readings.

‡Includes some pregnancies in which, because of early death of all embryos, there were no corpora lutea. In these cases the number of ovulated eggs was taken as 9.53, which is the mean number of corpora lutea per pregnancy for all pregnancies in which the corpora lutea were counted.

is clear, then, that so far as distribution of deaths with respect to age of embryos is concerned, there is no evidence of a qualitative difference in end results of the two experiments.

We can now turn to an examination of the results to see whether the quantitative response to neutron dose was different in cyclotron and detonation. This was considered the most important feature of the investigation when it was planned, and it was hoped that the physical dosimetry necessary for the comparison would, in spite of the difficulties of instrumentation under the field conditions, turn

Table 4.35—DOMINANT LETHALITY IN OFFSPRING OF MALE MICE MATED FROM 2 TO 6 DAYS AFTER EXPOSURE IN CYCLOTRON

| | | | Percentage of corpora lutea represented by | | | | |
|---|---|---|---|---|---|---|---|
| Dose, rep | No. of pregnancies | No. of corpora lutea | Living fetuses | Embryos or fetuses dying after day $10\frac{1}{2}$ | Embryos dying between implantation and day $10\frac{1}{2}$ | Eggs or embryos dying before implantation (100 minus sum of cols. 4, 5, and 6) | "Survivors" (sum of columns 4 and 5) |
| (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) |
| 0 | 20 | 192 | 79.69 | 2.60 | 6.77 | 10.94 | 82.29 |
| 47 | 10 | 92 | 53.26 | 1.09 | 20.65 | 25.00 | 54.35 |
| 56 | 13 | 125 | 48.80 | 0.80 | 25.60 | 24.80 | 49.60 |
| 82 | 12 | 125 | 44.00 | 0.00 | 19.20 | 36.80 | 44.00 |
| 99 | 10 | 92 | 40.22 | 0.00 | 27.17 | 32.61 | 40.22 |
| 116 | 8 | 77 | 36.36 | 0.00 | 28.57 | 35.06 | 36.36 |
| 141 | 5 | 45 | 22.22 | 0.00 | 40.00 | 37.78 | 22.22 |
| 160 | 10 | 93.7* | 25.61 | 2.13 | 33.08 | 39.17 | 27.75 |
| 194 | 6 | 66 | 27.27 | 1.52 | 21.21 | 50.00 | 28.79 |
| 209 | 5 | 52.7* | 22.77 | 0.00 | 28.46 | 48.77 | 22.77 |
| 252 | 4 | 38.7* | 10.34 | 0.00 | 25.84 | 63.82 | 10.34 |
| 257 | 2 | 20 | 10.00 | 5.00 | 45.00 | 40.00 | 15.00 |
| 305 | 1 | 9 | 11.11 | 0.00 | 22.22 | 66.67 | 11.11 |
| 311 | 1 | 9.7* | 0.00 | 0.00 | 30.93 | 69.07 | 0.00 |
| 369 | 0 | | | | | | |

*Includes one pregnancy in which there were no corpora lutea owing to early death of all embryos. In this case the number of ovulated eggs was taken as 9.7, which is the mean number of corpora lutea per pregnancy for all pregnancies in which the corpora lutea were counted.

out to be of an adequate degree of accuracy. It was planned to measure the total dose at each station with tissue-equivalent ion chambers and, by subtracting a separate measurement of the gamma component, obtain an estimate of the neutron dose. Measurements with ion chambers constructed of tissue-equivalent plastic were not feasible because of a limited number of chambers. Sheppard and Darden[1] designed and constructed a larger number of small tissue-equivalent ion chambers with polyethylene walls. These were suitable for measuring total dose over most of the range in which mice were to be exposed, and they performed better than anticipated. The readings obtained close to the mice inside the lead hemispheres at the various stations are shown in column 2 of Table 4.34. There is, unfortunately, some uncertainty as to whether ion collection in these instruments was complete under the high-intensity burst conditions.

Turning to the gamma component of the radiation, this is estimated from film dosimeter readings made by the Radiation Instruments Branch, Division of Biology and Medicine, U. S. Atomic Energy Commission. It appears, however, that the sensitivity of the films to neutrons is not negligible. At the present time it is possible to estimate the contributions of "gold" and "sulfur" neutrons to the film dosimeter readings. Subtracting these from the readings gives maximum estimates of the gamma radiation. These are shown in column 3 of Table 4.34. They are presumably considerably higher than the true gamma doses because no allowance has been made for the unknown, but possibly appreciable, contribution of neutrons of intermediate energies.

With these uncertainties about both the total dose and the gamma component at each station, how much can be said about the RBE of neutrons from the detonation as compared with neutrons in the cyclotron experiment? It turns out that if the current estimates of the total dose and the gamma component are used to compute the RBE of neutrons from the detonation, it seems safe to assume that the figure obtained will be a maximum estimate. This can be demonstrated in the following way: Consider first the effect of possible error in total-dose measurements. The most likely error in the ion chambers was incomplete ion collection. If, then, the true dose for a given biological effect was higher than recorded, the RBE would be less than that estimated from the recorded dose. Turning to the effect of error in the gamma measurement, it appears safe to assume that the film dosimeters picked up all the gamma radiation and that neutrons other than those with gold and sulfur energies may have had some effect. Thus the film dosimeter readings, even with the effect of gold and sulfur neutrons subtracted, cannot have underestimated the gamma radiation.

If the true proportion of gamma radiation for a given total dose was lower than that estimated, then the neutron dose must have been higher and, again, the RBE would be less than that estimated. To recapitulate: Physical measurements of both the total dose and the gamma dose may have been in error, but it appears likely that each could have been in error in only one direction, and it so happens that the direction in each case was such that a maximum estimate of the RBE of the neutrons can be calculated.

There remains the statistical problem of extracting the best point estimate and the confidence interval of this maximum value for the RBE. We are indebted to Dr. A. W. Kimball of the Mathematics Panel of ORNL for help in the solution of this problem. For statistical treatment, the biological effect is expressed as percentage survival through day $10\frac{1}{2}$ of gestation, column 10 of Table 4.34 and column 8 of Table 4.35. As was pointed out earlier, there is no apparent death from induced dominant lethals after that time in development. It is assumed that log survival is linearly related to dose. There are biological grounds for making this assumption, and the cyclotron and detonation data both give good fits on this interpretation. The relation of biological effect to dose can then be expressed in the following way:

Let $u_c$ = biological effect, cyclotron
$u_d$ = biological effect, detonation
$R_c$ = total dose (rep), cyclotron
$R_d$ = total dose (rep), detonation
$G$ = gamma dose, detonation
$B_c$ = RBE, cyclotron
$B_d$ = RBE, detonation

Then, assuming additive effects of neutrons and gamma,

$$\log u_c = \alpha_c + \beta(0.9R_cB_c + 0.1R_c)$$

since 10 per cent of the total dose in the cyclotron was found to be gamma radiation, and

$$\log u_d = \alpha_d + \beta[(R_d - G)\,B_d + G]$$

These equations can be rewritten as

$$y = a + bx_1$$

$$z = a' + b'x_2 + bx_3$$

where $y = \log u_c$ $\quad z = \log u_d$
$a = \alpha_c$ $\quad a' = \alpha_d$
$b = \beta$ $\quad b' = \beta B_d$
$x_1 = 0.9R_cB_c + 0.1R_c$ $\quad x_2 = R_d - G$
$x_3 = G$

By the method of weighted least squares, the two equations were fitted simultaneously to the values given in Tables 4.34 and 4.35. $B_c$ was taken as 8.0, the value obtained by comparing cyclotron results at the 100-rep level with data from an experiment with 250-kvp x-rays. A value of $B_c$ based on gamma radiation might turn out to be slightly higher, and $B_c$ might vary somewhat with dose. However, even considerable error in the estimate of $B_c$ will have little effect on the estimate of $B_d/B_c$. The estimated value of $B_d$, the maximum RBE of neutrons from the detonation, obtained from the

ratio of the estimates of b′ and b, is 9.44. Therefore $B_d/B_c$, the maximum estimate of the ratio of RBE of detonation and cyclotron neutrons, is 1.18. The 95 per cent confidence limits for this ratio are 0.91 and 1.55.

To obtain a minimum estimate of the ratio of the RBE of detonation and cyclotron neutrons requires an estimate of the maximum possible total dose and an estimate of the minimum possible gamma dose. Minimum gamma doses for each of the exposure stations are given by Butenhoff and Deal.[16] These are based on the attenuation through 7 in. of lead of the dose of gamma radiation recorded outside each lead hemisphere. The values are clearly minimum estimates of the true gamma doses inside the hemispheres, because they do not include any of the gamma radiation made by neutrons in the lead or inside the hemispheres.

Estimation of the maximum possible total dose presents more of a problem. It has been stated that there is some uncertainty as to whether ion collection in the ion chambers was complete under the high-intensity burst condition. Thus, the true dose may have been higher than that recorded in the ion chambers, but it is difficult to estimate how much higher. Fortunately, in detonation A it appears that ion collection may have been essentially complete. There are two independent reasons for drawing this conclusion: first, the parallelism of ion-chamber readings and sulfur neutron flux, as discussed in Chap. 2; and, second, the biological results reported here. By comparing the biological data with the cyclotron results, a dose estimate for each hemisphere was obtained in terms of the cyclotron dose in rep that would have produced the same biological effect. A least-squares fit of log (dose times distance$^2$) against distance was then made using these dose estimates. It is clear that this curve should have the same slope as one based on the true rep doses for the detonation, regardless of the RBE's and the gamma contaminations in the cyclotron and the detonation, provided that the RBE's are the same for different doses and that the gamma contaminations are constant proportions of the total dose. The slopes would be approximately the same without the RBE's being constant provided, as seems more likely, that the ratio of the RBE's is the same at different doses. The slope obtained using the biologically estimated doses is $-0.000617 \pm 0.000058$. Fitting the ion-chamber readings in the same way gives a slope of $-0.000611 \pm 0.000025$. It is clear that there is no significant drop in the ion-chamber readings at closer distances. This, then, provides evidence, additional to that obtained from physical considerations, that ion collection was essentially complete in the ion chambers used in detonation A.

A minimum estimate of the ratio of the RBE of detonation and cyclotron neutrons can therefore be obtained using the minimum estimates of gamma contamination and assuming the ion chambers did not underestimate the total dose. This was done with exactly the same method as that used for obtaining the maximum estimate. The estimated minimum RBE of neutrons from the detonation obtained by this procedure is 6.4, and the estimated minimum ratio of the RBE of detonation and cyclotron neutrons is 0.80, with 95 per cent confidence limits of 0.67 and 0.96. It should be kept in mind, however, that this minimum estimate is based on an assumption about the ion chambers that is not quite as secure as that used in calculating the maximum estimate.

Summarizing the results obtained in the attempt to discover whether the RBE of neutrons from the detonation differed from that of neutrons from the cyclotron, taking the RBE of cyclotron neutrons as 8, we have

| | RBE of detonation neutrons | RBE of detonation neutrons / RBE of cyclotron neutrons | |
|---|---|---|---|
| | | Point estimate | 95% confidence limits |
| Minimum estimate | 6.4 | 0.80 | 0.67 and 0.96 |
| Maximum estimate | 9.4 | 1.18 | 0.91 and 1.55 |

It is clear that detonation and cyclotron neutrons under the conditions of this test do not show a significantly different RBE.

It is perhaps of some interest to assume that the ratio of RBE is, in fact, unity, to assume that the ion-chamber measurements were correct, and then to see what percentage of the total dose has to be assigned to gamma contamination to give the observed biological results. Fitting the results to these assumptions gives a point estimate of the gamma component of 25 per cent, with 95 per cent confidence limits of 7.4 and 40 per cent.

In conclusion, it may be said that, so far as dominant lethals in mice are concerned, there is no evidence of qualitatively different effects of neutrons in the detonation and cyclotron experiments. There is also no evidence of a quantitative difference. Since the upper 95 per cent confidence limit of the estimated maximum ratio of RBE is 1.55, it seems safe to assume, as a starting point in the ex-

trapolation of mouse data to man, that it is unlikely that the hazard from the spectrum of energies and the intensity of the neutrons encountered in the detonation experiment is more than one and a half times that of the neutrons in the cyclotron experiment, and it may be no greater.

## 4.12 DEGENERATION OF SPERMATOCYTES AND TYPE "B" SPERMATOGONIA IN MICE

This study was a part of Project 23.13. Oakberg's preliminary report follows:

Mice to be used for measuring the histological changes induced in spermatogenic cells by neutrons released from a nuclear detonation were exposed in lead hemispheres arranged at various distances from Ground Zero of detonation A. Tissues were taken 48 hr after exposure. Paraffin sections of the testes were stained in hematoxylin-eosin for demonstration of necrotic spermatocytes, and by the PAFSA technique for enumeration of spermatogonia.

Table 4.36—NUMBER OF DEGENERATING SPERMATOCYTES OBSERVED 48 HR AFTER EXPOSURE TO NEUTRONS RELEASED FROM DETONATION A

| Station | Estimated total dose, rep | No. of tubules scored | Mean no. of degenerating spermatocytes per tubule | Fraction of spermatocytes surviving |
|---|---|---|---|---|
| 14 | 761 | 22* | 45.2 | 0.000 |
| 15 | 518 | 23 | 44.4 | 0.018 |
| 16 | 322 | 20 | 44.6 | 0.013 |
| 17 | 256 | 27 | 18.5 | 0.591 |
| 18 | 173 | 19 | 8.2 | 0.819 |
| 20 | 134 | 22 | 6.6 | 0.854 |
| 22 | 108 | 15 | 5.2 | 0.885 |
| 24 | 74 | 15 | 4.4 | 0.903 |
| 25 | 49 | 10 | 3.4 | 0.925 |
| 26 | 23 | 14 | 2.9 | 0.936 |
| Control | | 130 | 2.0 | 0.956 |

* As degeneration of spermatocytes occurs primarily at only one stage in spermatogenesis, only those tubules showing necrotic cells have been considered.

There is a regular increase in the number of degenerating spermatocytes with an increase in dose (Table 4.36). All cells irradiated as primary spermatocytes were destroyed by doses of 322 rep and above. The mean of about 45 degenerating cells per tubule therefore represents the maximum effect detectable. Likewise, 45 represents the effective cell population available for estimation of cell survival, and this value was used in converting the data to percentage of cells surviving radiation damage (Table 4.36). At present, only data from exposure to 600 r of x-rays are available for comparison, with a mean of 9.3 necrotic cells observed in 73 tubules. This one point indicates that 1 rep of neutrons from the nuclear detonation was approximately four times as effective as 1 r of x-rays.

Type B spermatogonia show a much higher induction of cell lethality than is obtained for primary spermatocytes. Loss of cells irradiated as type B spermatogonia is manifested as a deficiency in number of resting primary spermatocytes 48 hr later. Data given in Table 4.37 indicate surviving cells only at the lowest dose, which has been estimated as 23 rep. Preliminary data from exposure of mice to gamma rays confirm the high sensitivity of type B spermatogonia.

The results indicate that by using the biological effects on the testes reported here, two mice placed at each dose level would be adequate to estimate radiation damage for doses ranging from about 300 rep to less than 20 rep of neutrons. Experiments now in progress indicate that the lowest dose with an easily detectable effect probably will be in the range of 1 to 5 rep of neutrons.





[redacted]

Table 4.37—SURVIVAL OF TYPE "B" SPERMATOGONIA AS MEASURED BY OCCURRENCE OF RESTING SPERMATOCYTES 48 HR AFTER EXPOSURE TO NEUTRONS FROM DETONATION A

| Station | Estimated total dose, rep | No. of tubules | No. of cells expected | No. of cells observed | Percentage of expected no. which was obtained |
|---|---|---|---|---|---|
| 14 | 761 | 6* | 344 | 0 | |
| 15 | 518 | 9 | 516 | 0 | |
| 16 | 322 | 9 | 516 | 0 | |
| 17 | 256 | 13 | 745 | 0 | |
| 18 | 173 | 16 | 917 | 0 | |
| 20 | 134 | 13 | 745 | 0 | |
| 22 | 108 | 9 | 516 | 0 | |
| 24 | 74 | 20 | 1146 | 0 | |
| 25 | 49 | 8 | 458 | 0 | |
| 26 | 23 | 10 | 573 | 12 | 2.09 |

* Limited to only those tubules in which resting spermatocytes would be expected to occur.

Table 4.38—LITTER PRODUCTION BY PREGNANT FEMALES EXPOSED AT DETONATION A

| | Interval between copulation and exposure, days | | |
|---|---|---|---|
| | 1 to 6 | 6½ to 12 | Total |
| No. of females exposed | 19 | 25 | 44 |
| No. of litters carried to term | 2 | 16 | 18 |
| No. of litters available for study | 2 | 14 | 16 |
| No. of newborns available for study | 8 | 97 | 105 |

## 4.13 DEVELOPMENTAL EFFECTS IN MICE

This study was also a part of Project 23.13. The preliminary report by Dr. Liane B. Russell and Dr. W. L. Russell follows:

An attempt was made to study effects at birth of exposure to a nuclear detonation during the embryonic development of the mouse. For comparison with effects of other types of radiation, extensive data are available for x-ray[17] and for cyclotron neutrons (unpublished data of L. B. Russell).

Owing to the fact that this investigation had to be undertaken with little time for preparation, only 44 females (strain 101) that had copulated at appropriate intervals before detonation A were available. All of these were exposed in lead hemispheres at stations 24, 25, and 26. Because this number was low, it was thought that not enough animals could be spared for proper controls, which would have had to cover all the developmental stages available. This is unfortunate since extraneous factors may have been important in some of the results (e.g., yield of young). The females were returned to Oak Ridge on the day following detonation A and weighed daily until the expected date of parturition. The young were killed within 24 hr of birth, weighed, and measured. The external features and abdominal viscera were examined, and the animals were then processed for skeletal study.

Table 4.38 shows the number of litters and young available. The poor yield from females exposed in very early stages of pregnancy is comparable to x-ray findings, but the present result may be more extreme, possibly due to extraneous factors (connected with transportation of animals, etc.). Most data for the later period (days 6½ to 12), which roughly corresponds to the period of major organogenesis, come from animals exposed on days 9, 10, and 11 (a total of 92 young).

The skeletal study is not yet complete. Abnormalities noted externally and on examination of the viscera may be listed as follows (without percentage incidences, which vary greatly): anophthalmia, microphthalmia, open eyelids, brain hernia, harelip, short tail, abnormal tail, polydactyly, oligodactyly, syndactyly, abnormal leg torsion, abnormal spleen shape, small or imperforate anus, and horseshoe kidney. Our earlier x-ray data clearly indicated a definite dependence of type and incidence of a given abnormality on the stage and dose irradiated. This makes it possible to map "critical periods" for

Table 4.39—ABNORMALITIES FOUND AMONG LITTERS OF FEMALES EXPOSED TO DETONATION A

| | Station | | |
|---|---|---|---|
| | 26 | 25 | 24 |
| Estimated rep | 21.5 | 45.5 | 70.5 |
| No. of young examined | 27 | 64 | 14 |
| No. showing one or more abnormalities | 10 | 50 | 14 |
| Per cent showing one or more abnormalities | 37 | 78 | 100 |

each abnormality. The data from the detonation roughly fit in with these critical periods but do not provide a sufficient spread of stages, or sufficient numbers even within those stage-dose groups that are available, to be definitive or to state reliable RBE for various abnormalities. What may, however, be stated without doubt is that relatively low doses of neutrons from the detonation produced a high incidence of developmental abnormalities. Combining all stages, total incidences of abnormal animals (many of which have several different abnormalities) are shown in Table 4.39. These figures are based on external and visceral data only. The total incidences of abnormal animals will undoubtedly be greater when the skeletal data become available.

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CHAPTER 5

# DISCUSSION AND CONCLUSION

There are a number of conclusions which can be drawn from the results which do not depend upon accurate dosimetry. Thus Ives' observations of the relatively higher RBE for production of chromosome aberrations compared to mutations is of basic theoretical interest. That this is true in general seems well borne out in the data which are summarized in Table 5.1. In several instances stations in which different effects were investigated overlap.

The RBE for the aberration type of chromosomal disturbance tends to be higher than for mutations at a given set of stations. This is not surprising, perhaps, if we consider that the recoils from fast neutrons produce a high degree of disruption wherever they strike. That a greater number of breaks will occur simultaneously and that healing will be inhibited by the dense ionization seem not unreasonable. Further discussions of these ideas will be found in the literature.[1] If these differences are to be attributed to high ionization density, then the situation will be more pronounced for the highly degraded situation of the field experiments and for the cyclotron and reactor investigations than for a clean flux of monoenergetic neutrons in the 2- to 3-Mev range. However, the degraded neutrons are the most familiar ones to the health physicist.

The observation of Schwartz that at higher doses seedling height is greater is of passing interest and is independent of accurate dosimetry, since the closer-in stations will obviously receive more radiation and yet seedling height was, if anything, greater. Atwood was unable to find evidence of an oxygen effect on his *Neurospora* cultures. This is of interest since Stapleton[2] reports little if any effect on *E. coli* exposed to 0 to 40 krep of fast neutrons in the ORNL 86-in. cyclotron.

Of course, the important objectives of the test required physical dose measurement. Measurement of specific biological effects in lower forms of life alone at a specified distance (perhaps with a sulfur flux measurement) cannot give more than a hint of the expected hazard to man. If errors in physical dose are merely errors in scale magnitude, then the linearity of effect with dose which was observed (for example, in the *Drosophila* sex-linked lethal tests, Fig. 4.2) is unaffected. The nonlinearity of the *Mormoniella* mutations is somewhat unexpected since in *Drosophila* the linearity of mutation frequency with dose has been established with great care for x-rays and seems to exist also for fast neutrons. Where the log of lethal effect has been plotted against dose, the single-hit characteristics of the result seem, in general, to be confirmed (for example, in the *Drosophila* dominant lethals, Fig. 4.3). Since for x-rays the curves show some multiplicity effect and thus are not linear, the result is that the RBE varies with dose.

Comparison of effects in the ORNL cyclotron with field results requires merely that the dosimeters calibrated in the cyclotron read dose correctly under field conditions. The complicating effect of the probably greater gamma-ray contamination in the field and high-intensity burst conditions is the principal difficulty and does limit the precision of physical dose deter-

mination. Extrapolations of measured dosage into regions where dose could not be directly determined is particularly hazardous. In spite of this, however, the results are in fair agreement where field and laboratory tests were comparable. Perhaps these consistencies between biological and physical data, particularly in detonation A, are not fortuitous. If so, the physical studies benefited by the biological investigations. As Russell points out in Sec. 4.11, a maximum estimate showed that the field neutrons were not more than 1.18 times as effective as those in the ORNL cyclotron. The *Tradescantia* results and those of Lewis and of Stone indicate fair agreement. There were a few inconsistencies. Baker got good agreement for detonation A but poorer agreement (~60 per cent) for detonation B. Mickey and Yanders obtained poorer agreement also between cyclotron and detonation effects in their sex-linked lethal studies. The re-

Table 5.1—RBE PER UNIT DOSE OF FAST NEUTRONS COMPARED TO X-RAYS

| Project | Biological effect studied | Mutational* | Chromosome aberrational | RBE |
|---|---|---|---|---|
| 23.12 | *Neurospora* survival | | x | 2–5 |
| 23.1 | *Tradescantia* chromosomal aberrations | | x | 7–10 |
| 23.1 | *Tradescantia* chromatid | | x | 13 |
| 23.16 | *Datura* chromosome aberrations | | x | 15 |
| 23.4 | *Drosophila* sex-linked lethals | x | | 2 |
| 23.4 | *Drosophila* X-chromosome aberrations | | x | 8 |
| 23.6 | *Drosophila* mutations in chromosome III | x? | | 4.5 |
| 23.6 | *Drosophila* sex-linked recessive lethals | x | | ~2† |
| 23.6 | *Drosophila* dominant lethals | | x | 4.7–6.5‡ |
| 23.7* | *Drosophila melanogaster* rearrangements | | x | 5–7 |
| 23.7† | *Drosophila virilis* rearrangements | | x | 3–6 |
| 23.9 | *Mormoniella* eye-color mutants | x? | | 3–5 |
| 23.13 | Mouse dominant lethals | | x | 8 |

*May include some minute deletions in addition to mutations as taken in the strict sense.
†1.7 for detonation; 2.5 for cyclotron neutrons.
‡Varies with dose. Two-thirds as great in detonation B as A.

cent improvement of dosimetry by the Rossi-Failla group and the verification by Hurst and his associates of the utility of multiple fission and other threshold detectors should help materially to fill the existing gap in future experiments.

The validity of the absolute value of the cyclotron calibration in rep depends upon the validity of the tissue-equivalent ion chambers used. Some discussion of the method has recently appeared.[3] Since the same method was used in calibrating the Argonne fission source, the agreement of Lewis' bithorax dose estimates with the ORNL physical doses rests on the same foundation. Recent comparisons at Oak Ridge of the tissue-equivalent method with the Hurst proportional-counter neutron dosimeter in the ORNL cyclotron give good agreement between the two methods. In spite of these apparent consistencies, further work must be done to place neutron effects on a firm physical foundation. Such experiments are now in progress at Oak Ridge.

If the biological results are not in serious conflict with physical dose estimates and if biological neutron effects depend on neutron energy, then there cannot be a great difference in neutron spectrum in the field and in the Oak Ridge and Argonne facilities. It may, of course, be that highly degraded neutrons do not produce effects which are highly critical as to the neutron spectrum.

The most important result of the tests is the reconfirmation of the high RBE for some neutron effects (Table 5.1). Even for *Drosophila* gene mutations, the values are about 2. Previously it has been thought that the RBE for these effects was less than unity.[4]

That the RBE varies at different loci in *Drosophila* chromosomes is suggested by Mickey's findings. Unfortunately, it is difficult to accumulate enough mutations to provide good statistics; so the conclusions must only be accepted tentatively at the moment. In general, the high value for neutron RBE can be considered as again verified in this series of experiments and lends further support to the belief that, in the field, neutrons present a high potential biological hazard.

The results with *Neurospora* gave quantitative biological effects in the region up to 130 krep. Since this range extends to an estimated dose at 50,000 rep where physical dosimetry is still difficult, this material may be useful in future studies at high intensities. In the low-dose region, studies on spermatogonial degeneration in mice indicate little survival of type B spermatogonia at doses greater than 23 rep.

In conclusion, then, detonation neutrons, as seen through 7 in. of lead, did not differ very greatly in any observed way from laboratory-produced neutrons in their genetic and cytological effects as seen in plant materials, insects, fungi, and mice. Per unit dose, the ratio of neutron effects, relative to x-ray effects, varied from about 2 to 15 or more. Measurable neutron effects were produced from about 20 rep in mice as in lower forms of living material. The physical doses determined in outer stations and estimated by extrapolation inward were in rough agreement with biological results, but physical dosimetry was not entirely satisfactory and must be improved before further biological field tests can be contemplated.

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CHAPTER 6

# RECOMMENDATIONS

Genetic tests involving indexes of a wide range of neutron doses are those registering chromosome breakage in *Tradescantia* and *Datura* pollen cells and in the flies *Drosophila melanogaster* and *Drosophila virilis*. These take up little space and give invaluable supplementary data to the physical dosimeters. The sensitivity for dominant lethals in the mouse lies in between the sensitivities of the plant and insect materials, and reliable results were obtained with only 12 mice at each dose. Some of the foregoing organisms should be included in the dosimetry for future weapons tests. It is of especial importance that genetic test material should be exposed to devices of different types.

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